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THE DESOXYRIBONUCLEIC ACID CONTENT OF THE LIVER NUCLEI OF IMMATURE PULLETS TREATED WITH GONADAL HORMONES[1]

BY W. E. J. PHILLIPS[2], W. A. MAW[3], AND R. H. COMMON[4]

Abstract

Treatment of sexually immature pullets with estrogen, or with estrogen plus androgen, increased significantly the number of liver cell nuclei per kgm. live weight, but did not significantly affect the average amount of DNAP per nucleus (2.67×10^{-10} mgm. DNAP per nucleus). It is shown that increase in number of cells can account for approximately half the increase in liver weight evoked by estrogen or estrogen plus androgen. The rest of the increase may be in large part a consequence of cellular hypertrophy.

Introduction

Boivin, Vendrely, and Vendrely (2) originally suggested that the DNAP per somatic nucleus appears to be a constant characteristic of the species, in vertebrates at least, and that the diploid nucleus of the reproductive cell contains half as much DNAP. This observation was confirmed by Mirsky and Ris (15). Vendrely and Vendrely (18) showed that nuclei from avian species gave a compact group of values for DNA per nucleus distinct from the group of values for mammalian species. Since DNAP appears to be confined to the nucleus, similar considerations, therefore, apply to the somatic and reproductive cells themselves. Campbell and Kosterlitz (3) and Davidson (8) have submitted evidence that the DNA content of rat liver nuclei was not influenced by fasting or exclusion of protein from the diet. More recently Ely and Ross (11) have shown that the DNA content of the nuclei of cells from the rat's pancreas and small intestine was significantly increased by feeding a diet free from protein, even although the average volume of the nuclei was decreased by the protein-free diet. Campbell and Kosterlitz (4) subsequently pointed out that their results related to tissues of adult rats, whereas Ely and Ross (11) had worked with young growing rats. Campbell and Kosterlitz (4) have accepted the findings of Ely and Ross (11) and have ascribed the divergence between the two sets of results to differences in the

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responses of adult and of growing rats to protein deprivation. They have suggested, therefore, that liver DNA per nucleus may not be a suitable reference standard in growing rats for other cellular constituents liable to vary with alterations in dietary conditions, although it may be suitable in the case of adult rats.

Lecompte and de Smul (13) have extended the observations of Ely and Ross (11) to the hepatic cells of the rat. Others (17, 14) have shown that the volumes of the nuclei of rat liver cells form a geometric progression. Doubling of nuclear volumes is due to polyploidy (1). Harrison (12) demonstrated biochemically that 76% of the nuclei of the liver of the adult rat contained the tetraploid number of chromosomes while only some 20 to 30% were diploid. This observation agrees rather closely with the histological observations of Sulkin (17) and of McKellar (14).

It has been shown that the liver DNAP of the fowl, per kgm. live weight, is increased by estrogen (5, 7) although to a relatively smaller degree than is the liver RNAP per kgm. live weight. Since the liver cells of some vertebrates are known to exhibit polyploidy, this increase of DNAP might have been a consequence of hyperplasia or of increased polyploidy or of both causes. Accordingly the relevant observations have been repeated and at the same time extended so as to include counts of liver nuclei per kgm. live weight.

Experimental

Plan of Experimentation

Twelve crossbred (New Hampshire male × Barred Plymouth Rock female) pullets were assigned at random between four groups, each of three birds. The birds were fed identical amounts of a commercial developer ration, great care being taken to ensure that the food intake was the same for all birds on a given day.

The groups were treated with estradiol benzoate ("Progynon B", Schering) or testosterone propionate ("Oreton", Schering) by intramuscular injection according to the following scheme:

Group 1: Nil (sesame oil only).
Group 2: 2.0 mgm. testosterone propionate (TST) every other day.
Group 3: 2.0 mgm. estradiol benzoate (ODB) every other day.
Group 4: 2.0 mgm. TST + 2.0 mgm. ODB every other day.

The experimental period lasted 10 days. Five doses of hormone were given in all. On the morning of the 11th day the pullets were killed. The pullets were 42 days old at the outset and 52 days old at the end of the experiment. Subsequently a second lot of 12 pullets from the same hatching was taken and the experiment was repeated. The birds were 72 days old at the beginning of this second experiment and 82 days old at the end. The results of the two experiments displayed close agreement. Accordingly, the pooled results alone are presented in Table I.

General Experimental Technique

This has been described previously (7).

Analytical Methods

In some preliminary work considerable difficulty was experienced in securing consistent and reproducible counts of cell nuclei in fowl liver. Even the isolation of nuclei by Dounce's methods (9, 10) failed to yield consistent results owing to 'clumping' of nuclei. Part of the difficulty experienced with Dounce's methods may have been due to the fact that a refrigerated centrifuge was not available, and it was not possible to maintain a sufficiently low temperature by centrifuging in the cold room. Eventually a satisfactory modification of the method of Price and Laird (16) was used. Immediately after the birds had been decapitated and bled, the livers were excised, quickly weighed, and squeezed through the narrow orifices of a specially designed tissue pulper made of transparent plastic to the design of Dr. C. Allard, Notre Dame Hospital, Montreal, Que. This operation pulps the liver. The connective tissue is left as a thin pad between the perforated plate and the piston of the pulper. This preliminary separation of connective tissue makes it possible to effect subsequent homogenization with a minimum of mechanical treatment and consequent damage to the nuclei.

Approximately 1 gm. pulped tissue was weighed rapidly on a Roller Smith analytical balance, placed in a Potter–Elvehjem mortar tube with 5 ml. 0.25 *M* sucrose and homogenized for two and one-half minutes at 400 r.p.m.

TABLE I

EFFECT OF GONADAL HORMONES ON DESOXYRIBONUCLEIC ACID CONTENT OF NUCLEI OF LIVER CELLS OF THE SEXUALLY IMMATURE PULLET. DURATION OF EXPERIMENT, 10 DAYS. AVERAGE DATA FOR SIX BIRDS ON EACH TREATMENT

Group	1	2	3	4	LSD	
Treatment TST, mgm. ODB, mgm.	Nil Nil	5 × 2.0 Nil	Nil 5 × 2.0	5 × 2.0 5 × 2.0	$P = 0.05$	$P = 0.01$
Oviduct, gm.	0.14	0.25	7.2	14.2	—	—
Liver, gm. per kgm. live weight	17.7	17.6	32.2	28.0	4.5	6.2
DNAP, mgm. per 100 gm. liver pulp	35.1	36.8	27.2	27.3	3.2	4.4
Liver DNAP, mgm. per kgm. live weight	6.2	6.4	8.7	7.7	1.2	1.6
Nuclei per gm. liver pulp	13.0×10^8	14.3×10^8	10.0×10^8	10.2×10^8	1.5×10^8	2.1×10^8
Liver nuclei per kgm. live weight	232×10^8	252×10^8	317×10^8	287×10^8	51×10^8	69×10^8
DNAP per liver nucleus, mgm.	2.7×10^{-10}	2.6×10^{-10}	2.7×10^{-10}	2.7×10^{-10}	N S	N S

The homogenate was transferred to a 50 ml. volumetric flask and made to volume. This homogenate was diluted 1 + 4 with 1% orcein in 45% acetic acid. A drop was then placed in a haemocytometer (Neubauer ruling) and the numbers of nuclei in 25 large squares were counted.

The tissue was prepared for DNAP and RNAP determinations as described previously, except that pulped liver was used instead of untreated liver. Blocks were prepared by fixation in formol saline and dehydration in alcohol, followed by blocking and staining by normal routine procedure.

Experimental Results

The results are summarized in Table I. Estrogen produced changes of the expected order of magnitude in oviduct weight, liver weight, liver weight per kgm. live weight, and DNAP mgm. per kgm. live weight. These results confirmed previous work in this laboratory.

The major result, however, is the demonstration that the hormonal treatments did not alter significantly the average DNAP per liver nucleus. The average value for the 24 birds was 2.67×10^{-10} mgm. DNAP per nucleus.

This result can be seen also from the fact that the increases in DNAP mgm. per kgm. live weight are proportional to the increases in liver nuclei per kgm. live weight, as is set out in Table II. If the increase of liver weight evoked by estrogen had been due solely to uncomplicated hyperplasia, then estrogen could have evoked an increase of approximately 40% and estrogen + androgen an increase of approximately 24%. The relative increases in number of nuclei per kgm. live weight were of this same order. However, the relative increases in liver weight were roughly twice as great. This further increase of weight is probably due to cellular hypertrophy. Clavert and Randavel (6) have demonstrated histologically that the increase of weight of the liver of

TABLE II

CHANGES IN LIVER DNAP PER KGM. LIVE WEIGHT, IN NUMBER OF LIVER NUCLEI PER KGM. LIVE WEIGHT, AND IN LIVER WEIGHT PER KGM. LIVE WEIGHT, ALL EXPRESSED AS PER CENT OF THE CORRESPONDING VALUE FOR THE CONTROL PULLETS. AVERAGES FOR SIX BIRDS ON EACH TREATMENT

	Group			
	1	2	3	4
Treatment				
TST, mgm.	Nil	5 × 2.0	Nil	5 × 2.0
ODB, mgm.	Nil	Nil	5 × 2.0	5 × 2.0
Liver, weight per kgm. live weight	100	99	183	159
Liver, DNAP, weight per kgm. live weight	100	103	140	124
Number of liver nuclei per kgm. live weight (relative)	100	109	137	124

the female pigeon during reproduction is accompanied by both hypertrophy and hyperplasia. In the present experiment no such differences could be detected by examination of the histological preparations. It may be, however, that the technique was unsuitable for the detection of volume changes corresponding to changes of less than one quarter in linear dimensions.

These experiments have proved, firstly, that the hormonal treatments used did not affect the average amount of DNAP per nucleus in the immature pullet, and hence that the inferences drawn by Common, Chapman, and Maw (7) on the presumption of this fact were justified, the hormonal treatments used in both sets of experiments having been of the same order of magnitude; and, secondly, that approximately half of the increase in liver weight evoked by estrogen can be ascribed to hyperplasia. The experiments do not provide information on the DNAP per nucleus of avian liver tissue as compared with other avian somatic tissues, but this point is under investigation.

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A CYTOLOGICAL STUDY OF THE ANTERIOR SUBMAXILLARY GLANDS OF THE FOWL, *GALLUS DOMESTICUS*[1]

By D. J. McCallion[2] and H. E. Aitken[3]

Abstract

A cytological study was made of the anterior submaxillary gland of the domestic chicken at 1 day, 4 days, and 10 months of age. This gland is one of a number of aggregates distributed throughout the mouth and along the oesophagus. It is a compound tubular gland, whose ducts open in several places under the tongue. The glandular epithelium is a single layer of tall prismatic mucous cells. There are no serous cells. Most of the cells are loaded with mucus and the nuclei are flattened at the bases of the cells. The cells present various stages of secretory activity. No mitotic figures were seen. Tests for alkaline phosphatase were negative, but there was strong acid phosphatase activity. Lipase activity was slight but present. The Golgi material is composed of rods and granules lying between the nucleus and the lumen of the gland.

Introduction

Although the histology of the salivary glands of the chicken has been extensively described (Calhoun, 1933 (2)) and some cytological studies have been made on their secretory activity (Chodnik, 1948 (3)) there is no published account of any histochemical studies. This paper presents the results of some histological and cytological studies on the anterior submaxillary gland of the fowl (glandula inframaxillaris interna of Cholodowsky, 1892 (4)). In addition, the distribution of basophilia, mucus and mucopolysaccharides, acid and alkaline phosphatases, lipase, and glycogen were studied and their significance is discussed.

Material and Methods

The materials used in this study were the anterior submaxillary glands (Fig. 1) from a total of 22 birds, 1 day, 4 days, or 10 months old. The glands were exposed by cutting away the skin from the under surface of the head, dissected out, and fixed in the desired fixative.

For the demonstration of cytoplasmic basophilia, tissues were fixed in picro-alcohol-formol and stained in 0.2% toluidine blue. Companion sections were hydrolyzed for five minutes in *N* hydrochloric acid at 60° C. before staining. Both sets of sections were stained simultaneously.

The Golgi material was visualized by the DaFano technique. This method has been recommended for chick tissues by Hibbard (1942 (12)). Small pieces of the gland were fixed in cobalt nitrate – formalin and impregnated 48 hr. in silver nitrate solution. This was followed by reduction in hydroquinone mixture for 24 hr. The sections were toned in gold chloride solution.

For the identification of mucus, the glands were fixed in 4% basic lead acetate in 10% formalin and stained in 0.1% aqueous toluidine blue. Some sections were stained with 0.1% toluidine blue in 1% alcohol and in 30%

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alcohol. Tissues fixed in Bouin fluid or 10% neutral formalin were stained with mucicarmine. Sections from tissues fixed in picro-alcohol-formol were treated with periodic acid and Schiff reagent (McManus, 1946 (16), 1948 (17)). Control sections, previously treated in saliva at 37° C. for 15 min., were similarly treated.

For the demonstration of alkaline and acid phosphatases and lipase, tissues were treated according to the methods of Gomori (Lillie, 1948 (14), pp. 106-112). For alkaline phosphatase, tissues were incubated up to 40 hr. at 37° C. in sodium glycerophosphate solution buffered to pH 8.7. For acid phosphatase, sections were incubated for 36 hr. in a lead nitrate – sodium glycerophosphate solution buffered to pH 4.92. In both cases companion sections were incubated for the same times in buffered solutions without substrate. For the demonstration of lipase, sections were incubated for 14 hr. at 37° C. in a mixture of stearic hexitan ester (Tween 60) buffered to pH 7.3. Companion sections were boiled in water for nine minutes to destroy the enzyme prior to incubation in the Tween mixture. In all instances sections were counterstained with alum hematoxylin.

Tissues fixed in Bouin fluid or in 10% formalin were stained with Delafield's hematoxylin and eosin or Mallory's triple stain. All tissues were dehydrated through dioxane, except where special agents were required, and embedded in paraffin at 58° C. Sections were cut at 5 μ for all studies except those of the Golgi material and cytoplasmic basophilia when tissues were sectioned at 3 μ.

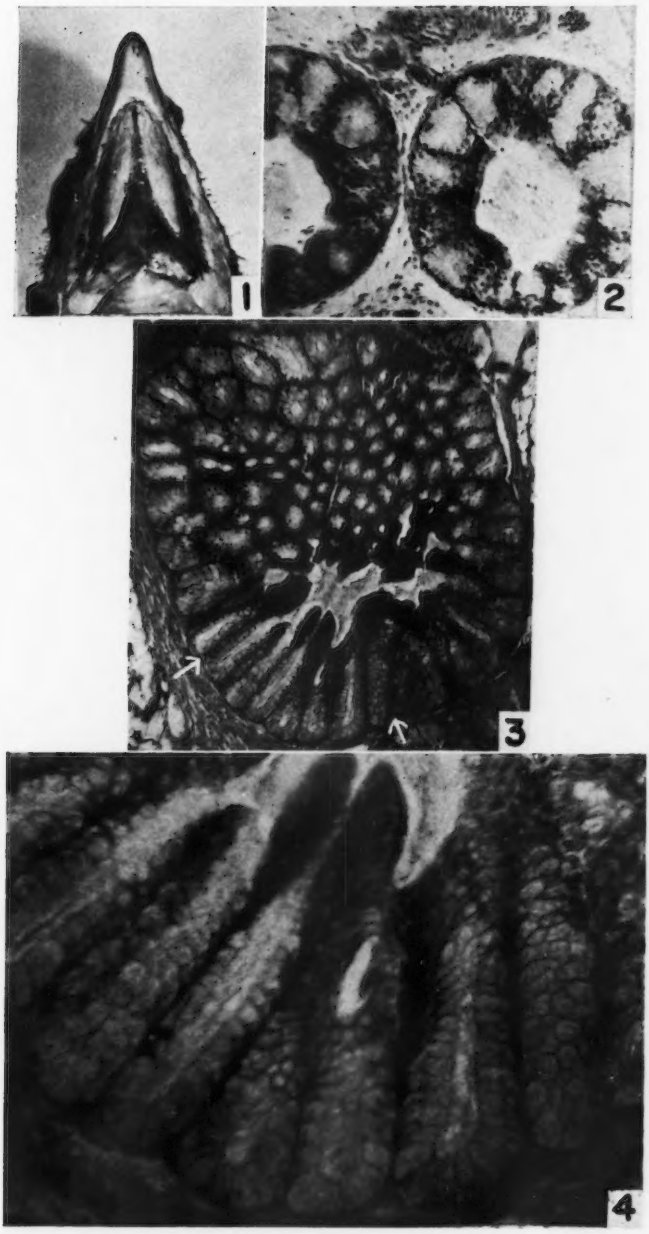
Results

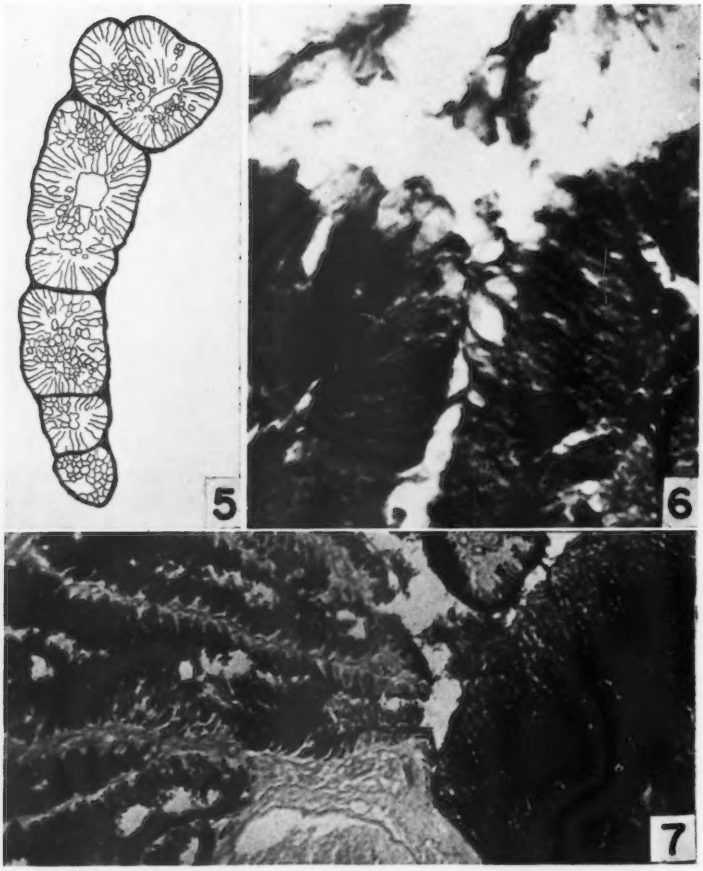
The salivary glands of the chicken form large and small paired aggregates distributed throughout the mouth and along the oesophagus (Cholodowsky, 1892 (4); Heindrick, 1908 (11); Calhoun, 1933 (2); and others). According to Calhoun the salivary glands of the chicken are all identical in structure. The anterior submaxillary glands are a pair of well-developed glands lying in the submucosa between the mandibles (Fig. 1). Each is a compound tubular gland, made up of three to four units in the chick, or many in the mature fowl. Each unit consists of many tubules having a common cavity and a common duct (Figs. 2, 3, 4). From the gland there are several openings into the mouth cavity, beneath the tongue. The entire gland is surrounded by a collagenous connective tissue capsule. Connective tissue separates the units and extends into the gland between the tubules of the unit (Fig. 5). Small blood vessels extend into the gland along with the connective tissue. Small

Figs. 1-4.

FIG. 1. A photograph (×1) of the ventral aspect of the dissected head showing the anterior submaxillary glands; age 10 months. FIG. 2. A section of the anterior submaxillary gland (×123) of a four-day-old chick showing its compound tubular structure. H & E. FIG. 3. A section of the anterior submaxillary gland (×74) of a 10-month-old bird showing its compound tubular structure. In the upper half of the section the small tubules have been cut transversely, and in the lower half longitudinally. Compare with Fig. 2. H & E. FIG. 4. A higher magnification (×290) of the area between the arrows in Fig. 3. Note that the tubules open into the lumen of the common duct. Compare with Fig. 2.

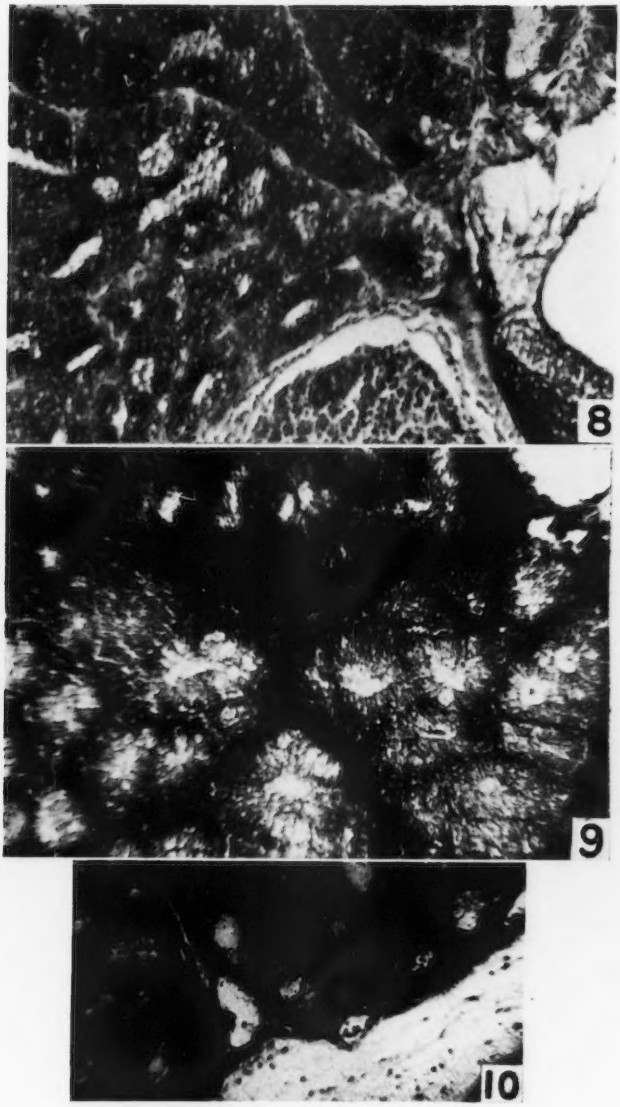
PLATE I





Figs. 5-7.

FIG. 5. A camera lucida drawing (×12) of a section of the anterior submaxillary gland. The black lines show the connective tissue. A connective tissue capsule surrounds the gland and separates the large compound tubes. Connective tissue trabeculae extend into the gland between the tubules of each unit. FIG. 6. A section of the anterior submaxillary gland (×500) of a 10-month-old bird showing rupturing cells and the secretory mass and cellular debris in the lumen of the gland. Compare with Fig. 4. FIG. 7. A section of the gland of a 10-month-old bird (×230) to show the dense staining of mucus in the glandular epithelium (left) and of the secretory product (right), after periodic acid-Schiff reagent. Note the lymphoid area, lower center.



Figs. 8-10.

FIG. 8. A section of the gland of a 10-month-old bird (×230) to show the dense basophilia of the glandular epithelium, secretory product (lower right), and lymphoid tissue (lower center). Toluidine blue. FIG. 9. A section of the gland of a 10-month-old bird (×150) to show acid phosphatase activity. FIG. 10. A section of the gland of a 4-day-old chick (×230) to show the acid phosphatase activity. Note the much greater density of enzyme activity compared with Fig 9.

islands of lymphoid tissue are scattered throughout the glands of older birds (Figs. 7 and 8), but are not seen in the glands of chicks. No muscle fibers (except those picked up from underlying tissue in dissection) or basket cells were observed in these glands.

The glandular epithelium consists of a single layer of tall prismatic cells that vary somewhat in size and shape with the stage of secretory activity and with their position in the tubule. Their nuclei are small, flattened, and lie close to the bases of the cells. The cells have a faintly marked border. No mitotic figures were ever observed, even at one day and four days of age. The tall prismatic cells of the glandular epithelium merge with the lower columnar cells of the collecting cavity. This low columnar epithelium abruptly meets the stratified squamous epithelium of the mouth cavity, which extends for a short distance into the duct.

The secretory epithelium of these glands is made up entirely of mucous cells. No serous cells were demonstratable. After treatment with periodic acid and the Schiff reagent a dense purple color was obtained, both in the glandular cells and in the secretory mass in the lumina of the glands (Fig. 7). No color was obtained in the lymphoid tissue of the gland. Companion sections previously incubated with saliva and similarly treated gave the same results, indicating that there is little or no glycogen present in these cells. The material in these cells also stained metachromatically in dilute toluidine blue. Mucicarmine, after several different fixatives, gave a positive test for mucus. No differences were detected between the glands of young chicks and older fowl.

The cytoplasm of the glandular cells was strongly basophilic (Fig. 8). The basophilic material appears as a dense cloud that is slightly darker in the distal portion of the cell than in the basal portion. The nucleoli and a perinuclear band are strongly basophilic. The lymphoid tissue and the secretory material in the lumen of the gland were also strongly basophilic. Treatment in *N* hydrochloric acid for five minutes completely abolished the basophilia in all of these sites.

At pH 4.92, acid phosphatase activity was quite strong (Figs. 9 and 10), but the enzyme activity was much greater in young birds than in the older ones. Acid phosphatase activity was strongest in the nuclei and in the basal portion of the cells, decreasing somewhat towards the distal end of the cells. There was very little acid phosphatase activity elsewhere in the glands, except in the walls of small blood vessels. No alkaline phosphatase activity whatever was detected. Some lipase activity was evident in the cytoplasm of the glandular cells and was slightly more marked in the glands of the older birds than in the chicks.

After the DaFano technique, the Golgi material varies from a small supranuclear network to many short rods and granules. The network configuration is rarely seen, and seems to be limited to small cells of irregular shape. The rods and granules are more or less concentrated near the nucleus, towards the base of the cell. This description applies equally to the glands of the chicken at all ages studied.

Evacuation of the cells seems to be accomplished by rupture of the distal end of the cell, allowing the contents of the cell to be discharged into the lumen of the gland (Fig. 6). When the cell has discharged its secretion it apparently disintegrates. In support of this observation many nuclei were seen in the secretory mass in the lumina of the glands. Although the animals used in this investigation were feeding ad libitum, very few cells were observed to be discharging their contents. In the glands of the older birds a higher proportion of cells were evacuating their secretion than in those of the chicks.

Discussion

Our description of the histological structure of the submaxillary gland of the chicken is essentially the same as that of Calhoun (1933 (2)). She described the oral glands as branched tubular glands and demonstrated that all the salivary glands of the fowl presented the same general histological structure. Although she reported muscle fibers in the salivary glands none were seen in our preparations, except for small masses of muscle picked up in making the dissections. In agreement with her, however, we also found no basket cells. Although Calhoun did not report the presence of small lymphoid masses in these glands, we found them distributed in the submaxillary glands of older birds, but not in chicks. It is possible, since these are present only in older birds, that these masses are ectopic lymphoid areas that are widespread among avian species (Lucas, 1951 (15)). It is generally agreed (Heindrick, 1908 (11); Calhoun, 1933 (2); and Chodnik, 1948 (3)) that the glandular epithelium of the oral glands of the fowl is composed entirely of mucous cells, and that none of the variety of types of cells commonly found in the salivary glands of mammals are present.

Both ribonucleoproteins and mucoproteins have a strong affinity for basic dyes and both are hydrolyzed by *N* hydrochloric acid (Dempsey and Wislocki, 1946 (7)). Basophilia that is abolished under these conditions may, therefore, be either ribonucleoprotein or mucoprotein. There is no doubt, however, that the material demonstrated in the fowl salivary gland is mostly mucoprotein. A strongly basophilic perinuclear band, observed in this study, is probably ribonucleoprotein and suggests that the synthesis of nucleic acid may take place at the nuclear membrane. Greenstein (1944 (9)) and others believe that a conspicuous nucleolus, such as we have described in these gland cells, is characteristic of cells that synthesize large amounts of protein.

Chodnik (1948 (3)) believed that after the cells ruptured and were evacuated, they became small, regenerated cytoplasm and organelles, and elaborated more mucus. It appears to us, however, that the cells completely disintegrate after rupturing at their distal ends, since the lumina of the glands usually contained considerable cellular debris and nuclei. Even though there were relatively few cells discharging their secretion, some new cells must arise by mitosis, but no mitotic figures have been observed by us or by previous investigators.

There are relatively few descriptions in the literature of the distribution of the phosphatases in the salivary glands of animals. A number of authors

(Gomori, 1941 (8); Kabat and Furth, 1941 (13); Deane, 1947 (6); and Bourne, 1943 (1)) are not in complete agreement. Noback and Montagna (1947 (18)) find that both acid and alkaline phosphatase reactions are present in the parotid, submaxillary, and sublingual glands of the rat and mouse with varying intensity of reaction. The alkaline phosphatase reaction is especially intense in the serozymogenic cells of the submaxillary gland. The absence of serous cells in the salivary glands of the fowl might explain in part the absence of alkaline phosphatase activity. The function of the phosphatases in the salivary glands is not known. However, if the phosphatases are implicated in the metabolism of nucleoproteins and nucleotides (Noback and Montagna, 1947 (18) and Dempsey and Wislocki, 1946 (7)) it is possible that they are implicated in the synthesis of the secretory product in the salivary glands of the fowl. If this were true it would explain the fact that the more dense acid phosphatase activity is in the basal part of the cell, where the product is presumably elaborated.

The Golgi system in various chicken organs (liver, Dalton, 1934 (5); developing stomach, Hibbard, 1942 (12); salivary and pancreatic tissue, Chodnik, 1948 (3)) has been generally described as a network. In various stages of secretion in the cells of the salivary glands, Chodnik also described lamellar spirals, threads, rods, and granules. In this investigation the network configuration was seen only in relatively few cells that were small and of irregular shape. In tall prismatic cells, presumably packed with secretory material, the Golgi system appeared as many short rods and granules. Previous studies of the Golgi material in the epithelial cells of the salivary glands of the fowl show that this organelle is associated with the production of mucus. Chodnik (1948 (3)) believed that the Golgi material was associated with the production of the secretory product, but he was unable to follow the transformation of osmiophilic granules to mucus vesicles. In a study of the Golgi–Thomas spheroidal system in the secretory cells of the palate gland of the chick, Grzycki (1950 (10)) found that various stages of the secretory cycle were expressed in variations in location and form of the Golgi system. Under the conditions of the present study it was not possible to relate distinctly the structure of the Golgi material and the secretory product, but we have no evidence contrary to that of Chodnik and Grzycki.

Acknowledgment

The authors are indebted to Dr. Wm. Montagna of Brown University for suggesting this investigation and for many helpful criticisms.

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POSTREDUCTION OF THE *X*-CHROMOSOME AND COMPLETE CHIASMA INTERFERENCE IN THE LAMPYRIDAE (COLEOPTERA)[1]

By Stanley G. Smith and Doreen E. Maxwell[2]

Abstract

Five species representing four genera in three of the five tribes comprising the Lampyridae of North America, the only genera known cytologically, have nine pairs of autosomes and an *XX* (♀) : *XO* (♂) sex-determining mechanism. A single individual of one species, *Pyractomena angulata*, carried supernumerary chromosomes, which varied in number in different cells from zero to four. The *X*-chromosome of males is strongly, positively heteropycnotic during early prophase; the *X*-bivalent of females is indistinguishable from the autosomal bivalents at pachytene. The complements of the five species are indistinguishable but within the complement there are considerable differences both in size and in the position of the centromeres. In all five species the chiasma frequency per cell is without exception nine: univalents not being encountered, the recombination index for the family, as known cytologically, stands uniformly at 81. Chiasma interference, being complete, must therefore extend across the centromere. The supernumeraries, which approximate one-half the size of the smallest regular autosome, have a chiasma frequency of *ca.* 0.25 per chromosome, or one-half that of the smallest autosome. They thus demonstrate that, contrary to the evidence provided by the regular complement, a length–frequency relationship exists among the latter but is masked by the totality of chiasma interference. As in other beetles, the centromeres are localized, but the lampyrids are unique in having postreductional division of the *X* in males. Other insect groups with postreduction have, or are reputed to have, diffuse centromeres: one, the Odonata, is invariably postreductional.

Introduction

In 1909, Stevens (32) recorded a difference in the division cycle of the *X*-chromosome in two species of Lampyridae that serves to set them apart from all other beetles known to possess an *XX*(♀) : *XO*(♂) sex-determining mechanism. The two have identical chromosome formulae, $9AA+XO$, as has a third species, *Lucidota* (= *Ellychnia*) *corrusca*, which Stevens studied (30), but which, in providing only spermatogonial divisions, failed to supply information as to the division sequence of the chromosomes during meiosis. The first-mentioned species were referred to the genus *Photinus* as *pennsylvanicus* and *consanguineus*, but in the generic disposition of the former Stevens proved to be in error, since this species rightly belongs in the genus *Photuris*.

At the time of her earlier publication, in 1906, Stevens described the behavior of the *X* in three other *XO* species of beetles; later (31) she dealt with three in the genus *Diabrotica* (one of which is now known as *Acalymma vittata*); and, concurrently with the account of *Photinus* and *Photuris*, she described and illustrated the sequence of events in three further *XO* species. In all nine the single *X* invariably segregated as a unit at first anaphase (prereduction) and underwent equational division at anaphase two. She accordingly made especial note (32, p. 104) of the fact that "These two species of

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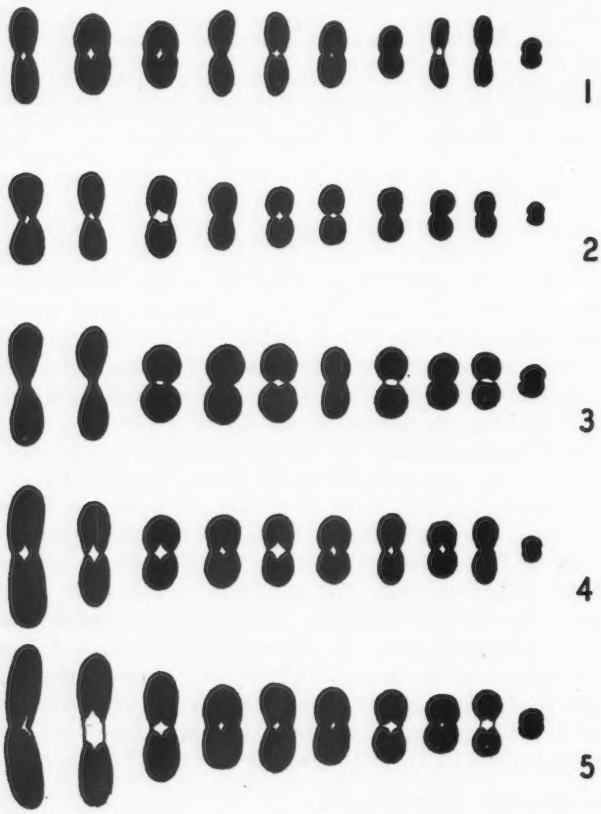
Lampyridae are the only cases which have been found, where the unpaired heterochromosome divides in the first spermatocyte instead of the second. In one, Photinus consanguineus, it divides very late, in a stage which is a late anaphase or telophase for the other chromosomes, while in the other species, P. pennsylvanicus, it divides at the same time with the other chromosomes, or only slightly later". Since the publication of Stevens' results, the total of *XO* species of beetles has been raised to 59, disposed among 29 genera in nine families (see compilation in Smith (28)) without a comparable case being described outside of the Lampyridae.

During the course of the past several summers, some 30 or so adult representatives of four genera of Lampyridae have been collected with the purpose of rechecking and furthering Stevens' observations and conclusions and of determining to what extent postreduction is a feature of the family. Pressure



FIGS. 1 to 5. Primary spermatocyte metaphases of *Photinus* sp., *Photuris pennsylvanica*, *Lucidota corrusca*, *Pyractomena borealis*, and *Pyractomena angulata*, respectively. For ease of comparison, the chromosomes have been aligned and spaced, with the univalent *X* placed at the right.

of other work has only recently permitted their detailed examination. It has, however, long been considered a pressing need because, coincident with the early discovery of Stevens' nomenclatorial error, it was noticed that *Photinus* and *Photuris* are placed in distinct tribes in Leng's (14) catalogue.

Henking (7) carried out an investigation of *Phausis* (= *Lampyrus*) *splendidula* but, since he was concerned only with females and failed to arrive at a satisfactory chromosome determination, his results will be omitted from consideration.

Materials and Methods

The four genera studied in the present paper include the three that Stevens examined and a fourth, *Pyractomena*. These and the other component genera of America, north of Mexico, are disposed in tribes as follows, in the arrangement adopted by Leng:

Lamprocerini — *Pleotomus*, *Calyptocephalus*, *Matheteus*, *Ginglymocladus*
Lucidotini — *Lucidota*, *Tenaspis*
Photinini — *Pyractomena*, *Photinus*
Lampyrini — *Microphotus*, *Phausis*
Photurini — *Photuris*

All individuals, identified by kindness of Mr. W. J. Brown, Systematic Entomology, Science Service, Ottawa, were studied as usual in squash preparations fixed in Smith's (23) modification of Kahle's fluid and stained in leucobasic fuchsin and light green (16 min. hydrolysis at 60° C.; *ca.* 2 hr. Feulgen). Camera lucida drawings were made at an original magnification of about 6600 diameters. Photomicrographs were taken on contrast process panchromatic film and developed in D 11; positives were printed in Kodabromide F3 and F4 paper. All drawings and photomicrographs are reproduced at approximate magnifications of ×5133 and ×2340 diameters, respectively. We are indebted to Mr. D. C. Anderson for assistance with the photomicrographs.

Observations

Photinus sp.

A single male, which could not be identified as to species, provided only diplotene and later stages, but these were of extraordinary beauty and occurred in great abundance. The diploid chromosome number, as in all male lampyrids except one specimen of *Pyractomena angulata* Say, is 19. Late diplotene (Fig. 6) and diakinesis (Fig. 7) nuclei contain nine bivalents and a univalent *X*-chromosome, which is positively heteropycnotic at the earlier stage. Chiasmata between the autosomal homologues are originally interstitial, with a maximum of one per bivalent, and terminalization is completed before prometaphase. During diplotene and early diakinesis the *X* is frequently associated with one of the smaller bivalents, but the association never persists after late diakinesis.

At first metaphase (Figs. 1 and 8) the unpaired *X* is as small as, or perhaps a little smaller than, the smallest autosome. The double structure of the component chromosomes may then still be partly revealed, so that the bivalents appear quadripartite and the *X* bipartite. This feature, together with the relatively greater size of the chromosomes at this stage, serves to differentiate first from second metaphases, which is otherwise difficult owing to the remarkable similarity of the two (see especially Figs. 22 and 23 of *Pyractomena borealis*).

The *X*-chromosome divides during the first division when the autosomes are in late anaphase (Fig. 9). Consequently, second metaphases are all of one type with nine autosomes and an *X* (Fig. 10). Unlike the autosomes, the *X*-chromosome passes undivided to one or the other pole at the second anaphase, with the result that two types of telophase nuclei are produced, one with 9, the other with 10 chromosomes (Fig. 11).

Photuris pennsylvanica (DeG.)

Several adult males of this species were dissected but only two were young enough to provide stages as early as diakinesis. At that stage there are nine autosomal bivalents and a lone *X*-chromosome, again quadri- and bipartite respectively (Fig. 18), the latter here again being associated with one of the small bivalents but separating before first metaphase (Fig. 2). In agreement with Stevens, this is a species, like the foregoing, in which the *X* undergoes postreduction; it is accordingly unipartite and present in all second metaphases. Segregation without division again leads to the production of equal number of telophase nuclei with 9 and 10 chromosomes.

Lucidota corrusca (L.)

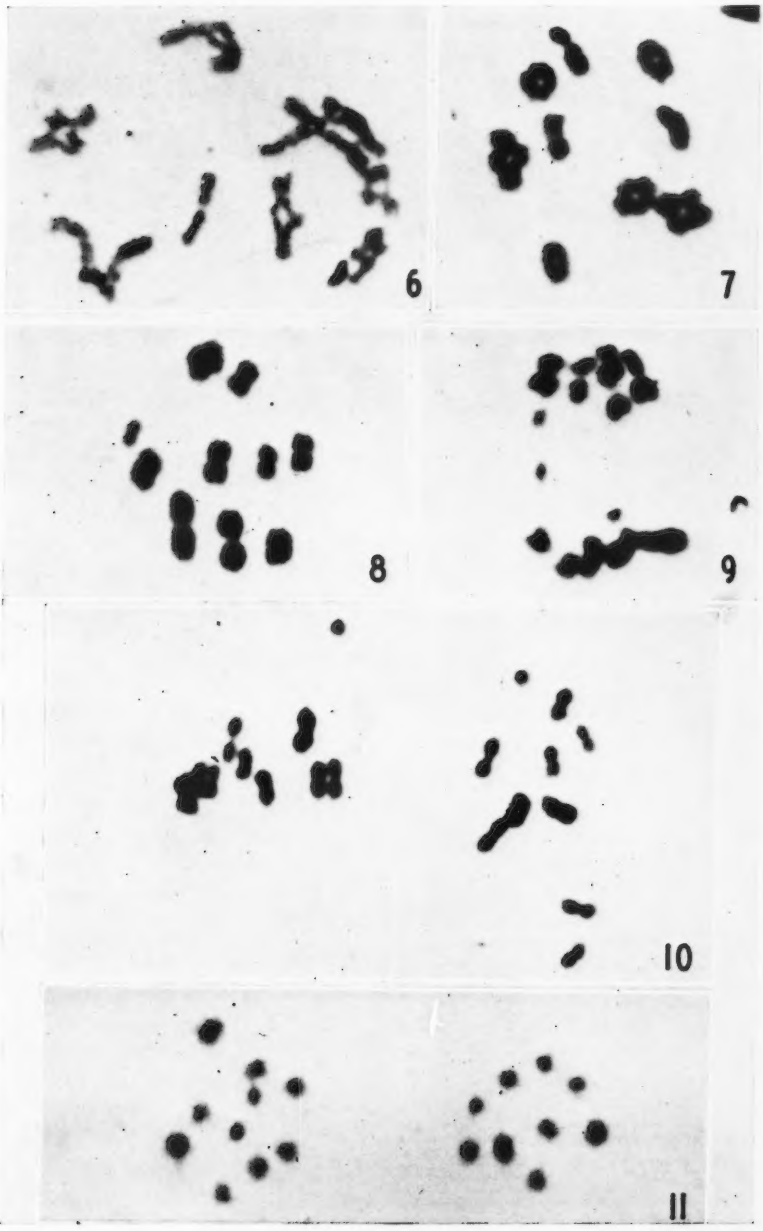
The genus *Lucidota* is recognized by Green (5) as consisting of a complex of types that have never been properly tabulated, so that until this has been done the correct designation for certain components cannot be determined. Our material consists of a dozen or so specimens, all of which key out close to Linnaeus' *corrusca* and, since they show no cytological differences, they might with impunity be grouped under this name.

Both males and females provided gonial metaphases; those of the former have 19 chromosomes (Fig. 12) and occur together with all later stages up to mature sperm; those of the latter have 20 chromosomes (Fig. 13), with later stages only as far as pachytene.

FIGS. 6 to 11. Spermatogenesis in *Photinus* sp.

FIG. 6. Late diplotene showing nine bivalents with a single chiasma each, all but one being unterminalized; the *X* is attached terminally to one of the smaller bivalents. FIG. 7. Diakinesis; again each bivalent has a single chiasma and the *X* is still attached to one pair. FIG. 8. Early first metaphase; the bivalents are quadripartite and the *X*, which has now dissociated, is bipartite. Terminalization is complete in all but one bivalent. FIG. 9. First anaphase with the *X* divided but lagging behind the autosomes. FIG. 10. Two second metaphases, each showing nine bipartite autosomes and the unipartite *X*-chromosome. FIG. 11. Two second telophases with 10 chromosomes (left) and nine chromosomes (right).

PLATE I



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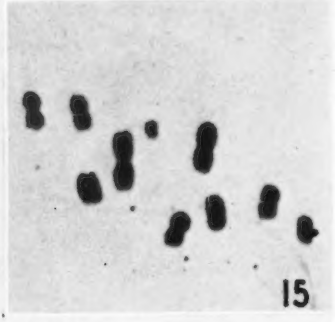
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Stevens' (30 and 32) illustration of a spermatogonial metaphase in "*Ellychnia corrusca*" is in full agreement with that observed in our males (Fig. 12) in having one pair of long rods, one pair of large V-shaped chromosomes, and 15 relatively shorter chromosomes with submedian to almost terminal centromeres. She labelled as *X* one of the longer of the short chromosomes, but we have been unable to find any feature by which one chromosome could be so specified.

In males the *X*-chromosome is condensed and somewhat strongly positively heteropycnotic at pachytene, but whether it is associated with the nucleolus, as in so many other species of beetles, could not be determined. On the other hand, in females the *X*-bivalent is indistinguishable from the autosomal ones (Fig. 14)—all bivalents are fully euchromatic, with their component chromosomes intimately paired throughout their length. The nucleolus was unstained in the females also, so that whether it is free or united with the *X*-bivalent remains an open question. Unfortunately, later oocyte stages failed to stain with Feulgen.

Bivalents at diplotene and early diakinesis in spermatocytes comprise a variety of configurations, from pairs in almost terminal association at one extreme to simple, symmetrical open crosses at the other. These latter show that association, as in the other species examined, is always by means of a single interstitial chiasma. Progressive terminalization during diakinesis is completed by prometaphase, at which time the bivalents are indistinguishable from the *X* in their staining capacity, although the latter is readily picked out by being smaller and by being bipartite instead of quadripartite.

For a brief period after early first metaphase (Fig. 15), the chromatids become intimately associated so that the *X* assumes an almost spherical shape and the bivalents appear bipartite (Fig. 3); then they 'relax' and again reveal their dyad and tetrad nature. The bivalents are remarkable in their apparently uniform shape: they are all quadripartite and differ only in size, despite the two largest being, one, acrocentric and, the other, mediocentric and the smaller ones differing considerably in the position of their centromeres. It is clear that the constituent chromatids of each homologue and of the *X*-chromosome must be somewhat independently spiralized and already to some degree dissociated in preparation for their eventual separation. This occurs during

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FIGS. 12 to 17. Gonial and meiotic stages in *Lucidota corrusca*.

FIG. 12. Spermatogonial metaphase with $2n = 19$ showing the great range in the size of the chromosomes and the varied positions of the centromeres; the *X*-chromosome is not distinguishable. FIG. 13. Oogonial metaphase with $2n = 20$ showing the size and shape-range of the chromosomes; the two *X*-chromosomes cannot be distinguished from the autosomes. FIG. 14. Pachytene in a female illustrating the indistinguishability of the *X*-bivalent from the nine autosomal ones. FIG. 15. Early first spermatocyte metaphase; the autosomal bivalents have one terminal chiasma each and still show their quadripartite nature, the *X* is faintly bipartite. FIG. 16. Two first anaphases in a male; in one the chromatids of the *X* are just separating; in the other, which is somewhat later, they are well apart (cf. Fig. 9). FIG. 17. Three second spermatocyte metaphases; each shows nine bipartite autosomes and a unipartite *X*-chromosome, which in the middle cell is superimposed on an autosome.

the first anaphase in the case of the *X*, after the autosomes are already well disjoined (Fig. 16), but is deferred until the second anaphase where autosomes are concerned.

As in *Photinus* and *Photuris*, postreduction ensures that all secondary spermatocytes are of one type. At prometaphase the autosomes are bipartite and, with the exception of one, appear to consist of two acrocentric chromatids joined in the form of a dumbbell; the exceptional one has its chromatids lying side-by-side on the equator. The *X*, of course, is a unipartite sphere and generally lies off the plate. At full metaphase (Fig. 17), the contiguous chromatids of the exceptional chromosome "unfold" and fall into line with the spindle, after which all the daughter chromosomes separate to opposite poles, one lot being accompanied by the undivided *X*. Late anaphases and telophases therefore have 9 and 10 chromosomes.

Pyractomena borealis (Rand.)

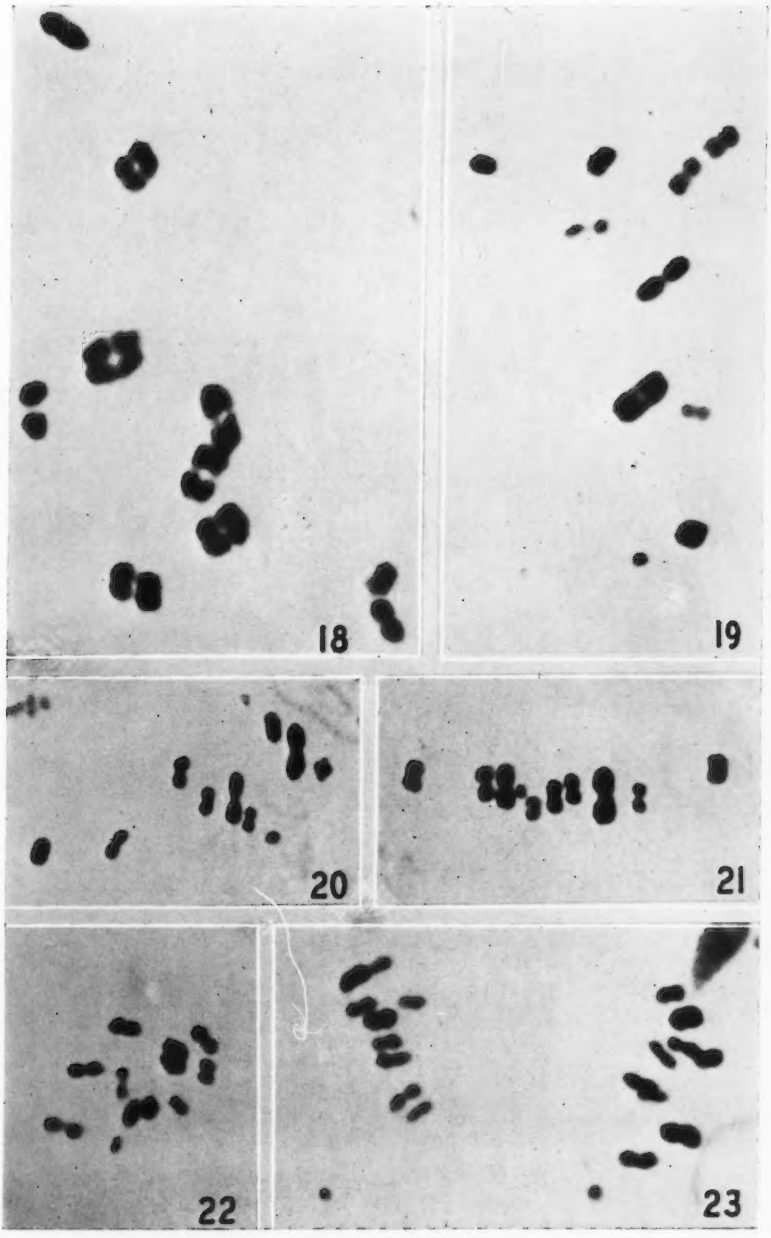
Several adult males of this species were collected, but they provided only stages later than diplotene. These stages closely resemble those of the species already dealt with: a chiasma frequency of one per bivalent; terminalization usually completed by prometaphase (Fig. 19); quadripartite bivalents and a bipartite *X*-chromosome, which transitorily become bipartite and unipartite respectively (Figs. 4, 20, and 21); a first anaphase equational for the *X* only; uniform second metaphases (Figs. 22 and 23); and a reductional second division culminating in the production of the two expected numerical classes of second telophases.

Pyractomena angulata (Say)

With the exception of one individual, this species is indistinguishable in the number, morphology, and behavior of its chromosomes from the foregoing species (see Figs. 5, 24, and 25). The abnormal specimen has a higher and variable chromosome number (Figs. 26 and 27): it carries supernumerary chromosomes, the presence of which is readily detectable at late diplotene and diakinesis in primary spermatocytes and, with greater ease, at full metaphase. The inclusion of these extra, small chromosomes, smaller even than the small *X*, renders difficult a precise determination of the role played by the *X*-chromosome as a reductional or equational component of the first anaphase nucleus.

FIGS. 18 to 23. Spermatogenesis in *Photuris pennsylvanica* and *Pyractomena borealis*.

FIG. 18. Late diakinesis in *P. pennsylvanica* showing the bipartite *X* still in association with one of the nine bivalents all of which have a single, terminal chiasma. FIG. 19. Prometaphase in *P. borealis*; note that the basic chromatid structure is shown only by some of the larger of the nine bivalents and that pairing in all is through a single chiasma, which is terminal in all but one. FIGS. 20 and 21. First metaphases in *P. borealis* showing the size range of the bivalents, the close association of their chromatids and those of the *X*, and the complete terminalization (except for one bivalent in Fig. 20). FIGS. 22 and 23. Secondary spermatocytes at prometaphase and metaphase in *P. borealis*: at prometaphase, the chromatids of the largest autosome are still lying side-by-side; at metaphase, they assume an end-to-end orientation before separating.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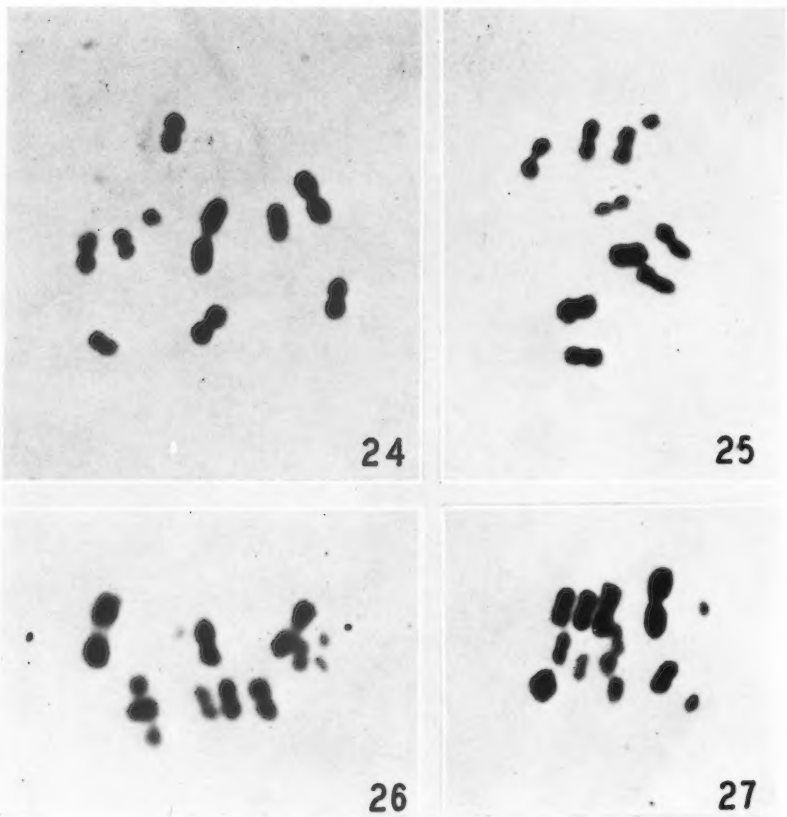

FIGS. 24 to 27. Meiosis in *Pyractomena angulata* males without and with supernumerary chromosomes.

FIGS. 24 and 25. A first and second metaphase in a 19-chromosome individual: in Fig. 24, the nine bivalents each have a single, terminal chiasma and the basic chromatid structure of both the autosomes and the *X* is concealed; because of this, first metaphases are indistinguishable, except by the greater size of the components, from the second metaphase in Fig. 25. FIGS. 26 and 27. First metaphases from the male that had different numbers of chromosomes in different cells. FIG. 26. shows the nine bivalents and univalent *X* of the regular complement and a small supernumerary bivalent to one side; FIG. 27 has a supernumerary univalent (lying to one side with the *X*) as well as the extra bivalent, which lies near the middle.

It is assumed, however, with a high degree of certainty, that its division sequence in this individual parallels that established for the *X* in 19-chromosome males of this and the other species.

The supernumerary chromosomes were found to vary in number from zero to four per nucleus. Where two or more were present they showed a capacity for associating in twos (Figs. 26 and 27), but trivalents and quadrivalents were not seen (Table I). The number of complexes resolvable at diakinesis is limited by the initial variability in number of supernumeraries and the possibility that at this stage they share with the *X*-chromosome the property of sometimes revealing and sometimes concealing their basic chromatic constitution, two factors which together militate against a satisfactory analysis of the pairing relationships and the frequency distribution of supernumeraries. This holds not only for diakinesis but also for second metaphases, for after division the *X* is indistinguishable from the supernumerary chromosomes.

On the premise that double spheres at diakinesis are supernumerary bivalents rather than univalents in which the paired chromatids are visible, 100 cells at diakinesis and metaphase were examined to assess the frequency of those having zero to four extra chromosomes and to determine their pairing behavior. As will be seen from Table I, the sample established the mean

TABLE I

THE FREQUENCIES OF CELLS AT DIAKINESIS AND METAPHASE WITH VARIOUS NUMBERS AND ASSOCIATIONS OF SUPERNUMERARY CHROMOSOMES

Stage	0	1_I	2_I	1_{II}	3_I	$1_I + 1_{II}$	4_I	Total numbers of		
								Cells	Supers	Xta
Diakinesis	4	2	3	12	1	0	0	22	35	12
Metaphase	6	6	22	27	6	10	1	78	156	37
Total	10	8	25	39	7	10	1	100	191	49

number at 1.91 per nucleus. If the associated supernumeraries are considered to be held in pairs by chiasmata, the total number of chiasmata, 49, is distributed between the 183 supernumeraries, that occur in cells containing two or more, with a frequency of *ca.* 0.25 chiasmata per supernumerary chromosome.

The frequencies of cells with from zero to four supernumeraries are compatible with the interpretation that the individual originally possessed two extra chromosomes and that a lack of co-ordination with the other chromosomes during spermatogonial mitoses led to their numerical increase and decrease in different daughter cells. Since the preparation was made by squashing, it has proved impossible to check the validity of this suggestion.

Discussion

A comparison of Stevens' (32) figures of an oogonial and a spermatogonial metaphase in *Photinus consanguineus* illustrates well the inadvisability of attempting to identify homologous chromosomes on the basis of size and shape, as does also her fig. 2 of an oogonial metaphase in *Photuris pennsylvanica*, in which one of the two largest chromosomes is a rod and the other a V. Experience with a considerable number of Coleoptera has made us reluctant to employ a process of elimination by picking out supposed homologues in identifying the *X*-chromosome in spermatogonial metaphases. Contrary to the claims of Goldsmith (4) and Minouchi (16), among others, we feel convinced that the *X* can be labelled with complete assurance, at least in beetles, only in those cases where it possesses morphological attributes, such as those in *Luperus discrepans* Baly and *Luperodes praeustus* Mots. (35 and 36) or *Tribolium confusum* Duv. (25 and 27), which immediately and irrevocably distinguish it from the other components.

Unfortunately gonial mitoses were present in only one of the species examined. Stevens experienced the same difficulty and overcame it by collecting larvae in October and keeping them indoors, whereby she was able to make dissections of larvae and pupae at progressively later dates. Immature specimens not being available, and their determination in any case being open to question, we are unable to compare the gonial complements of the various species investigated, so that, with the exception of *Lucidota*, it has not been possible to correlate the spermatogonial chromosomes with those of the first metaphase. It nevertheless seems probable, as is shown by comparing Stevens' (32) illustrations of *Lucidota* ("fig. 1") and *Photinus* ("fig. 22"), that the karyotypes of the various species are markedly similar. Judging, then, from first metaphases, it seems that there is no considerable morphological difference between *Lucidota*, *Photuris*, and *Photinus*. Possibly the two *Pyractomena* species are distinguishable from the others in the greater size of the largest bivalent.

The discrepancies between Stevens' and our figures of first metaphases in particular are readily attributable to our use of more modern techniques and this undoubtedly accounts for the nondescript appearance of the bivalents as she depicts them.

That several of the smaller rod-shaped chromosomes in *Lucidota* are actually acrocentric rather than telocentric was established by the observation of extremely minute second arms at early anaphase in spermatogonia. Others are nevertheless metacentric, as the large V most certainly is.

Ignoring the single individual that carried supernumerary chromosomes, the Lampyridae share a property, uniformity of karyotype, with only the

Dermestidae and Cleridae of the 33 families now studied cytologically. Moreover, of those families of beetles where more than one species has been examined, the lampyrids alone consist solely of *XO* species. This is somewhat surprising, for they constitute one of the more primitive of beetle families, with a fossil record dating back to the Lias. They have therefore been exposed to the possibility of numerical and structural change over a considerably longer period of time than have many other families that exhibit an extreme complexity of karyotypes. Since such changes have apparently not materialized, a cytosystematic consideration of Green's (5) recent revision of the Nearctic genera of Lampyridae is effectively precluded. Some considerations have been presented in earlier papers (26 and 27) which point to paracentric blocks of heterochromatin as playing a fundamental role, through segmental interchange, in the origin of the numerical changes that characterize so many coleopteran families. It is therefore perhaps significant that these blocks have not so far been detected in any of the species of Lampyridae we have examined.

The presence of supernumerary chromosomes in beetles has previously been recorded only by Stevens (31) in "*Diabrotica 12-punctata* and *D. soror*" (now *D. u. undecimpunctata* Mann. and *D. u. howardi* Barber, respectively) but it seems highly probable that they occur also in *Altica chalybea* (32) and *Zabrotes subfasciata* Boh. (16). According to Makino's (15) list of animal chromosome numbers, additional chromosomes of this type are restricted to the Insecta and, therein, occur predominantly within the orthopteroid complex of orders. With the exception of the grasshopper *Camnula pellucida* (2) and an undetermined Indian species of the gryllid genus *Euscyrtus* (29), extra chromosomes in the orthopteroid complex are, like the *Diabrotica* supernumeraries, numerically constant for the individual but variable for the species. This intraspecific inconstancy is readily attributable to recombination following the loss or gain of chromosomes at meiosis. In *Euscyrtus* most of the variability between individuals is clearly the result of the random distribution of supernumeraries, for these never pair, but premeiotic loss (or gain) of a chromosome is definitely established as a secondary cause in one individual. *Pyractomena angulata* accordingly stands in a position intermediate between that of the *Diabrotica* species and the Orthoptera in general, on the one hand, and that of *Euscyrtus*, on the other, for the numerical variability of the individual, and hence of the species, stems from both premeiotic loss or gain and meiotic failure of chiasma formation.

That supernumerary chromosomes are well adapted to play a fundamental role in producing evolutionary changes in chromosome number is generally accepted. A similar function is attributed to the paracentric blocks of heterochromatin so frequently encountered in both the Plant and Animal Kingdoms—see, for example, Babcock (1) for accounts of Dubinin's experiments with *Drosophila* and Tobgy's with *Crepis*. Since

supernumerary chromosomes and paracentric heterochromatin seem logically equated, it is almost incongruous that the former but not the latter occur in the Lampyridae, especially in view of the otherwise exceptional uniformity of the family in both chromosome number and morphology.

In *Photinus*, *Pyractomena*, and *Lucidota* it is readily established that the terminal associations of metaphase are relics of interstitial chiasmata that have been terminalized during the course of the preceding stages. In earlier publications Smith (24 and 25) has admitted that the use of the term "chiasma" was merely as a convenient substitute for the purely descriptive expressions "association" or "union", for in the beetles there discussed no direct evidence was found that the associations were derived from exchanges between pairs of paired chromatids. This, of course, is due to the components of the bivalents being joined at, or very close to, their ends even as early as diplotene and, in those cases where they are more median in position, to the impossibility of resolving the complexes into their constituent chromatids.

The diplotene and diakinesis stages in lampyrids in which terminalization is as yet incomplete bear a striking resemblance to those of the Acrididae that possess acrocentric chromosomes, such as species of *Melanoplus* and *Chorthippus*. The lampyrids, moreover, parallel other Orthoptera in the genus *Stethophyma* (=*Mecostethus*) in that despite the substantial range in the length of their chromosomes (the longest is some four times the length of the shortest in spermatogonial metaphases) they nevertheless have a consistent frequency of one chiasma per bivalent, or 0.5 per chromosome. The absence of univalents therefore establishes this value as both the upper and lower limits set by the genotype: variance is zero or, in other words, interference is complete and invariably operates across the centromeres. Unlike that in *Stethophyma* and also in *Tribolium confusum* (25), the interference here does not imply a localization at formation. Although it is perhaps understandable that the number of chiasmata in rod bivalents should be limited to one—a low chiasma frequency seems to prevail in beetles (but see Virkki's treatise on Scarabaeidae, (33))—it is surprising that the large V of *Lucidota*, for example, was never seen to form a ring. In *Tribolium* the absence of chiasmata from relatively short arms was shown to be correlated with an insufficient length of euchromatin, most of the length of the arm being occupied by paracentric heterochromatin; as has already been stressed, such blocks of heterochromatin have not been found in lampyrids. It has not, however, proved possible to determine whether it is invariably the same arm of the V that is involved in chiasma formation.

It is interesting to note that the similarity of these genera, representative of three of the five tribes comprising the North American Lampyridae, is not confined to the equivalent morphology of their chromosomes but is extended to include identical chiasma frequencies. Having the same chromosome number and a rigid chiasma frequency of one per bivalent, all the cytologically known species thus have an invariable recombination index of 81. As Darlington (3, p. 77) has stated ". . . for any particular inter-mating group there must

at any particular time be an optimum amount of recombination". Herein we see for the first time recorded a whole family (so far as is known) uniformly adapted to the lowest amount compatible with regular pairing.

It is unfortunately not possible to determine the precise size of the supernumerary chromosomes relative to that of the regular autosomes, but it might perhaps approximate one half that of the smallest autosome. Since the latter has a chiasma frequency of 0.5, the observed value of *ca.* 0.25 chiasmata per supernumerary implies that the length of the shortest autosome is the minimum compatible with orderly chiasma formation in the regular complement. The existence of such an intrinsic length-frequency relationship is masked in regular autosomes by the interference factor. It is apparently to this factor, as well as to their small size, that the failure of the supernumeraries to form multivalent configurations must be attributed. Since *P. angulata* is adapted only to accommodate chromosomes down to a certain minimum length, approximating that of the shortest member of the regular complement, it follows that, compared with the other components, the supernumeraries are indeed newly acquired: they will be subject to continuous numerical change in succeeding generations until such time as a genotype is evolved which will ensure their regular pairing.

The frequent attachment of the *X*-chromosome to one of the smaller bivalents is a phenomenon earlier recorded in various Odonata by Oksala (17). The two differ, however, in degree, for whereas in the Lampyridae the *X* is invariably fully independent by earlier metaphase, in certain of Oksala's *Aeschna* species the association not only persists throughout male meiosis but actually precedes meiosis, the *X* being attached to an autosome in spermatogonial and even in somatic cells. In Orthoptera such associations are found to involve autosomal heterochromatin (cf. Janssens' "dyade compagnon de l'X" in *Mecostethus* (13)) and are always of only temporary duration at prophase, thus paralleling those in the lampyrids rather than the extreme species of Odonata. In the lampyrids, however, heterochromatin is apparently not involved, for heteropycnosis, which distinguishes heterochromatin in most Coleoptera (25, 26, 27, and 33), is absent from the autosomes.

As mentioned earlier, Stevens was much impressed by the fact that although the *X*-chromosome divides at the first anaphase in both *Photinus* and *Photuris* its timing is relatively later in the former. Anaphase being absent from our *Photuris*, it has not been possible to verify this, but there is no notable difference between our *Photinus* and *Lucidota* (Fig. 9 vs. Fig. 16). In agreement with Stevens, we note that postreduction is strictly anticipated by the *X*: in species of beetles characterized by prereduction, the *X* looks unipartite during metaphase; in these, with postreduction, it is largely bipartite. Clearly the difference in the division sequence of the sex chromosomes is correlated with a difference in the division cycle of the centromeres: only where prereduction obtains is the centromere of the *X* synchronized with those of the autosomes.

A difference between species in the division sequence of the *X* is unknown in two of the major groups of insects that are typified by *XO* sex-determining

mechanisms, namely, the Orthoptera and Odonata, but the former is invariably a "prereductional" order and the latter a consistent "postreductional" one. A situation diametrically opposed to that in Coleoptera is, however, recorded by Hughes-Schrader (11) for the Coccoidea. There, the *X* divides at the first and segregates at the second anaphase in the whole of the tribe Llaveiini, whereas a reversed sequence is found in just one species, *Matsucoccus gallicola*, the only member of its subfamily so far examined. It is unfortunately difficult, owing to the peculiar "chromatid autonomy" found in this group, to ascertain whether the outward appearance of the *X* gives any indication of its subsequent behavior. A similar anomalous division of the *X* is shown in the Heteroptera, where *Archimerus calcerator* (34) and species of *Pachylis* (19 and 20) alone show prereduction. The question of the sequence of division in the Hemiptera as a whole has recently been dealt with in some detail by Helenius (6).

The intensive studies of Heteroptera and Coccoidea by Schrader (21) and Hughes-Schrader (8, 9, and 10) respectively have established beyond reasonable doubt that these two groups are characterized by having diffuse, rather

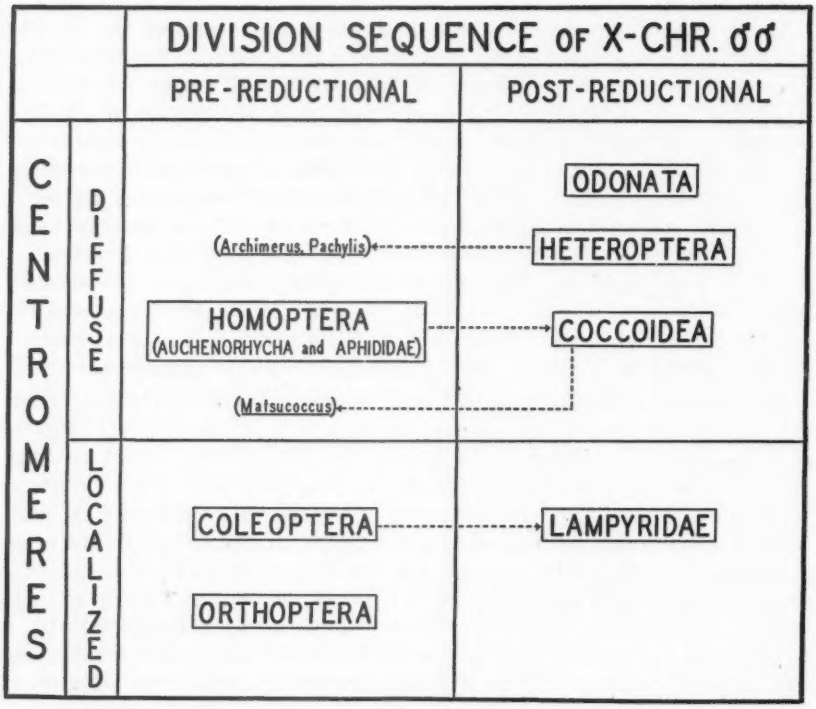

FIG. 28. Diagram showing the distribution of the reversal in division sequence of *X* and its correlation with diffuse and localized centromeres.

than localized, centromeres, and the experimental attack on the problem by Hughes-Schrader and Ris (12) has proved the interpretation to be correct, at least so far as the scale insect *Steatococcus tuberculatus* Morr. is concerned. Recently, Schrader (22) and Hughes-Schrader (11) have broached the possibility that the chromosomes of Odonata are structurally similar. This view has more recently been challenged by Oksala (18). If, however, the American workers are correct, it is a remarkable fact that these three groups, the Coccoidea, Heteroptera, and Odonata, are also characterized by postreduction of the *X*, although only the last mentioned is invariable for this property. The lampyrids are therefore at present unique among Coleoptera in being the only known instances where the reverse change, from pre- to postreduction, has occurred; in fact, with the status of the Odonata questionable, they constitute the only known examples of such a reversal among organisms with conventional localized centromeres (Fig. 28). It is consequently not a mere coincidence that this reversal involves all the cytologically known species in one family—the change doubtless occurred but once and preceded evolutionary divergence within the Lampyridae.

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THE LIMNOLOGY OF AMETHYST LAKE, A HIGH ALPINE TYPE NEAR JASPER, ALBERTA[1]

By D. S. Rawson

Abstract

Amethyst, at an altitude of 1965 m. (6450 ft.), is one of the few lakes in the Canadian Rockies investigated limnologically. The annual ice cover persists for nearly eight months, and the highest mean lake temperature is about 10° C. The water is clear, low in mineral content, and well oxygenated. Plankton and bottom organisms are of few species and present in quantities within the lowest range for oligotrophic lakes. The lake appears to be typically alpine oligotrophic, of a group characterized by some European workers as panoligotrophic. The only fish present is the rainbow trout, introduced in 1931. They grew rapidly, then deteriorated, and later made a slow recovery. The third generation has lost some of the characteristics of the parent stock *Salmo gairdnerii kamloops*, and begins to resemble the high mountain subspecies *S.g. whitehousei*.

Introduction

Although the beauty and the angling attraction of the high lakes of the Canadian Rockies have long been known, their possibilities in the field of limnology have been little explored. Our knowledge of the faunal characteristics and of the distinctive oligotrophy of high alpine lakes is largely derived from the studies of Pesta, Steinböck, and other European workers. Valuable work has been done in the high alpine lakes of western United States, listed by Pennak (7), but most of this has been related to fisheries studies rather than to fundamental limnology.

Amethyst Lake near Jasper is of special interest as a high, clear lake and one into which a lowland stock of rainbow trout has been introduced successfully. Our first observations were made in 1939, during a limnological survey of the area for the National Parks Bureau. In 1946, a grant from the National Research Council made it possible to carry on a full summer's observation at the lake. In July, 1952, the writer made another brief visit to the lake and examined a sample of the trout population.

The Geographic Situation

Amethyst Lake lies about 15 miles (24 km.) southwest of Jasper in the Tonquin Valley and an equal distance southeast of the Yellowhead Pass. The latitude is 52° 42′ north, the longitude 118° 16′ east, and the altitude 6450 ft. (1965 m.). It is sheltered to the west by a magnificent series of peaks, the Ramparts, which rise to about 9800 ft. (2995 m.) (Fig. 1). Much of the valley may be described as alpine meadow, but on its eastern slope a thin forest, mostly of Engelmann spruce, extends to an altitude of about 6700 ft. (2040 m.). The treeline in the vicinity lies generally between 6000 and 7000 ft. (1830–2135 m.).

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Contribution from the Biology Department, University of Saskatchewan, Saskatoon, Sask.

The lake drains south and east into Astoria Creek and the Athabaska River, but much of the Tonquin Valley drains north through Meadow Creek. Thus the drainage basin for Amethyst Lake proper is extremely small, about 26 km.^{2} (10 sq. mi.), and less than six times the area of the lake. This drainage area is made up of the west slope of Mount Clitheroe 9014 ft. (2790 m.) and the precipitous east face of the Ramparts. There have been no detailed geological studies in this area but the rocks are known to be mostly of Cambrian age, and to consist mainly of relatively insoluble quartzitic sandstones.

The location of the lake is such that the major glaciers of the area do not drain into it. Three small glaciers on the east slope of the Ramparts contribute to its water supply but most of the inflow is filtered through masses of fine deposits. Two small streams which originate on Mt. Clitheroe and drain into the east side of the lake are usually clear. Thus the lake water is relatively clear and free of silt through most of the season.

The mean annual air temperature at Jasper is 1.1° C. Making allowance for the difference in altitude of more than 900 m., the probable mean annual temperature at Amethyst Lake is about −8° C. The mean annual precipitation at Jasper is 30.5 cm. (12 in.). The much-studied lakes of the European Alps lie in a region where precipitation is four to eight times that of the Jasper region. The lakes studied by Hutchinson (3) at high altitudes in Tibet are at the other extreme, in an area with an annual rainfall of about 9 cm. (3½ in.).

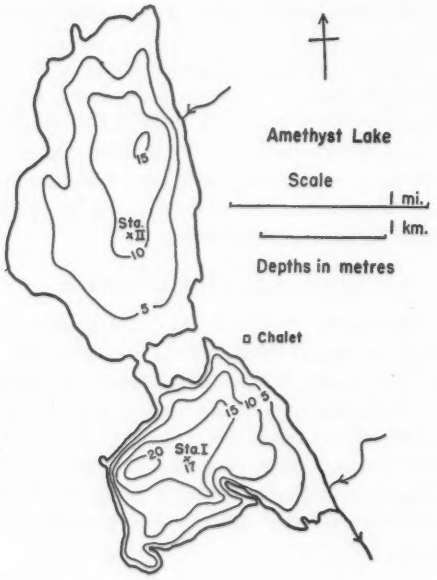

FIG. 2. Map of Amethyst Lake showing depth contours and location of stations.

PLATE I

FIG. 1. Amethyst Lake from the summit of Mt. Clitheroe (9014 ft.) looking west toward the Ramparts. Compare with Fig. 2. (Photograph by Department of Mines and Technical Surveys, Ottawa.)

Morphometry

The lake consists of two basins united by a narrows about 50 m. wide and 2 m. deep, Fig. 2. The north basin has an area of 296 ha., a maximum depth of 15.5 m. and a mean depth of 5.9 m. The area of the south basin is 174 ha., its maximum depth 21 m., and its mean depth 9.7 m. The total area is thus 470 ha. (4.7 km.^{2} or 1.8 sq. miles). The combined mean depth is 7.3 m. but this figure has little significance since the two basins have different thermal characteristics. The volume of the lake is 34.3×10^6 cu. m. The relative areas of the depth zones and volumes of the depth strata are recorded in Table I.

TABLE I

RELATIVE AREAS OF THE DEPTH ZONES AND VOLUMES OF THE DEPTH STRATA IN THE TWO BASINS OF AMETHYST LAKE

Depths, meters	Area of depth zones as percentage of total area		Volumes of depth strata as percentage of total volume	
	South basin	North basin	South basin	North basin
0– 5	23.6	48.6	45.4	64.0
5–10	28.1	34.6	32.0	28.7
10–15	29.4	16.4	17.3	7.3
15–20	17.2	0.4	5.3	—
20–21	1.7	—	—	—

The shoreline has a length of about 45 km. (9 miles). Most of the south basin has rough rocky shores but near the mouths of the inflowing creeks there are small areas of sand. The shores of the north basin have several stretches of narrow sandy beach. Rooted aquatic vegetation is scanty but in sheltered areas there are beds of a broad-leaved pondweed, *Potamogeton praelongus* Wolf, and a dwarf sedge, probably *Juncus* sp. In some places the bottom has coverings of *Nitella* sp. which extend to a depth of at least 5 meters. In other places there is a bottom mat of *Cladophora fracta* and *Ulothrix zonata*.

The bottom deposits in the open water area were described as a soft silt, usually gray, but often with yellow or reddish tints. Traces of organic matter were noticed and the deposits were quite unlike the very fine-grained, light gray substance on the bottom of heavily silted lakes.

Physical and Chemical Conditions

The thermal cycle of Amethyst Lake is noteworthy for its period of ice cover of seven and one-half to eight months. Freezing over usually occurs between November 1 and 15 and thawing from June 15 to July 1. The maximum thickness of the ice is reported as 46 cm. (18 in.). In 1946 the ice was gone from the shallower north basin on June 19 and two days later from the south basin. By June 29 the surface temperature at Station I was 6.4° C. and at bottom (15 m.) 4.6° C. The complete temperature curve for this date is shown on the graph, Fig. 3.

The summer temperatures for the south basin are outlined in Table II. The surface reached a maximum of 12.1° C. in mid-August and the highest bottom temperature (at 16.5 m.) was 6.8° on August 3. The highest mean temperature was on August 19. The complete series of observations on this date are plotted in Fig. 3. The mean temperature was 9.9° C. for the south and 11.6° for the north basin. The shallower north basin warmed more rapidly than the south. Vertical series taken at Station II on the dates indicated in Table II showed temperatures in the north basin to be higher than those at similar depths in the south basin by from 0.8° to 2.8°. The temperature series for September 6, 1939, corresponds to a mean temperature of 9.3° C. This would suggest that some cooling had occurred and that the mean temperature of 9.9 on August 19, 1946, was near the maximum.

The summer heat income of the south basin to August 19 was 5650 gm-cal. per sq. cm. This may be compared with 7550 gm-cal. from Bow Lake (mean depth 17.6 m.) and 8900 gm-cal. for Maligne Lake (mean depth 40.5 m.) (9), and to 5800 gm-cal. for Flakevatn (mean depth 30 m.) in Norway, studied by Strøm (17). Amethyst is obviously within the range for high alpine lakes with regard to its heat income. Bow Lake has a greater heat income than Amethyst because of its greater depth but it is actually the colder of the two. The highest mean temperature of the upper 10 m. in Bow Lake is 9.1° C. in contrast to 11.4° for Amethyst Lake (both basins).

The graph, Fig. 3, shows an obvious thermocline between 11 and 14 m. on August 19. There was no thermocline in the north basin and it was observed in the south only in the late season and in the deeper area. This stratification is thus limited to a small portion of the lake area and has little effect on the trophic situation. The dissolved oxygen of deep water never fell below 75% saturation. The presence of a thermocline is of special interest in view of the controversy between Steinböck (14) and Pesta (8) as to its occurrence in lakes

TABLE II

LIMNOLOGICAL OBSERVATIONS AT STATION I, 17 M., IN THE SOUTH BASIN OF AMETHYST LAKE, 1946

	June 29	July 3	July 19	Aug. 3	Aug. 19	Sept. 6*
Temperature, ° C.	Surf. 6.4	6.7	10.9	11.0	12.1	10.0
	5 m. 5.6	—	10.3	10.8	10.8	10.1
	10 m. 5.0	5.9	7.7	8.6	10.3	9.4
	Bott. 4.6	5.7	6.5	6.8	4.4	5.8
Dissolved oxygen, mgm./liter	Surf. 9.3	9.3	8.3	8.1	7.4	7.7
	Bott. 9.2	9.3	9.0	9.2	7.9	8.1
pH	Surf. —	—	—	7.1	7.2	7.2
	Bott. —	—	—	7.1	7.2	6.9
Secchi disk, m.	7.5	8.7	9.5	11.0	8.5	4.5

* *1939.*

of a kind which the latter described as panoligotrophic. It should be noted, however, that the thermocline in Amethyst Lake was in a deep position (11-14 m.) while Steinböck emphasizes the "epilimnial" thermocline as characteristic of alpine lakes.

The amount of dissolved oxygen in surface and bottom samples throughout the season is indicated in Table II. These values must be interpreted with reference to the observed temperatures and to the high altitude of the lake. The observations range from 7.4 to 9.3 mgm. per liter. This, at an altitude of 1965 m., is equivalent to 9.5 and 11.9 mgm. per liter at sea level. The lowest saturation value at Station I is 77%, found at 17 m. on August 19. On the same date a water sample from the greatest depth, of 20 m., had 7.6 mgm. per liter dissolved oxygen, equivalent to 74% saturation. The dissolved oxygen was always higher in the north basin than in the south. On August 19, Station II, at 10.5 m., the saturation value was 86%. Thus it is clear that the lake is at all times well oxygenated, even in the small deep region beneath the thermocline.

The hydrogen ion concentration of the water was measured at a pH of 7.1 or 7.2. On September 6, 1939, the deepest water had a pH of 6.9. Thus the normal condition of the lake may be said to be slightly alkaline.

The transparency of the water measured with a Secchi disk showed a gradual increase from 7.5 m. in June to 11 m. in early August, then some reduction in

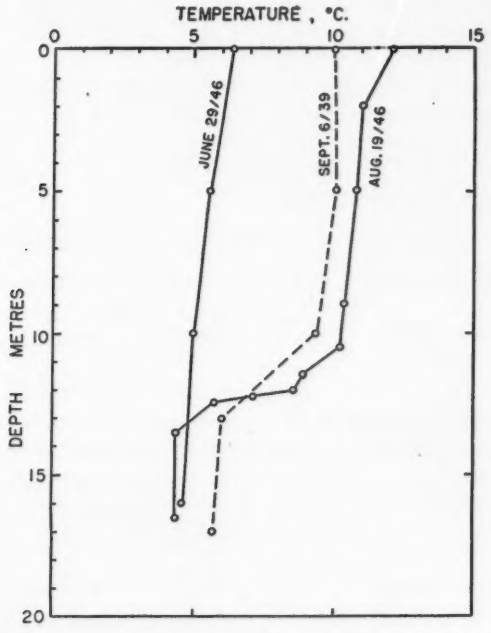

FIG. 3. Temperature curves at Station I, Amethyst Lake, 1939 and 1946.

late summer. This is very clear water for an alpine lake among glaciers. Such transparency could only exist where most of the flow from the glaciers is well filtered through intervening deposits before reaching the lake. By way of contrast, Bow Lake, of similar size and altitude, is fed directly from a glacier and has transparencies of 0.4 to 1.5 m. (9).

The mineral content of Amethyst Lake water was determined for a surface sample taken in late July, 1952. The total dissolved solids by evaporation at 110° C. was 38 p.p.m. The materials in the residue were determined as follows:

Bicarbonates (HCO_3), 34.6 p.p.m.	Calcium (Ca), 4.9 p.p.m.
Sulphates (SO_4), 9.2 p.p.m.	Magnesium (Mg), 3.5. p.p.m.
Chlorides (Cl), —	Sodium (Na), 5.2. p.p.m.

The total mineral content, 38 p.p.m., is very low but not surprising in view of the small drainage area and hard rock surroundings.

The Plankton

Plankton collections were made along with the physical and chemical observations at Stations I and II. A large closing net of No. 20 silk with a mouth 25 cm. in diameter was used to take total vertical hauls and surface tows. These were supplemented with a few stage hauls to determine vertical distribution and a number of qualitative hauls in shallow water. All samples have been examined for species identification. Duplicate total vertical hauls at each station allowed one sample to be used for dry weight and ash determination while the other was used for counting.

The phytoplankton, identified by the Reverend P. Kuehne, Annaheim, Sask., included representatives of at least 20 genera and a slightly larger number of species. Most of these species were found only in the shallow inshore collections. The phytoplankton of the open water was very sparse. The list of phytoplankters is as follows:

Cyanophyta

Coelosphaerium kuetzingianum Naeg.

Chlorophyta

Eudorina elegans Ehr.
Oedogonium sp.
Dictyosphaerium pulchellum Wood
Pediastrum boryanum (Turp.) Menegh.
Oocystis borgei Snow
Scenedesmus arcuatus Lemm.
Zygnema sp.
Spirogyra sp.
Cosmarium botrytis Menegh.
Hyalotheca dissiliens (Smith) Breb.

Chrysophyta

Tabellaria fenestrata (Lyngb.) Kuetz.
Navicula spp.
Stauroneis parvula var. *prominula* Grun.
Cymbella aspera (Ehr.) Cleve
Amphora caffaeiformis (Ag.) Kuetz.
Epithemia argus Kuetz.
Epithemia sorex Kuetz.
Surirella elegans Ehr.
Campylodiscus hibernicus Ehr.

Pyrrophyta

Ceratium hirundinella (O.F.M.) Schrank

The dominant phytoplankters of the open water were the diatom *Tabellaria* sp. and the desmids *Hyalotheca* and *Cosmarium*. *Tabellaria* was most abundant on July 3 whereas *Hyalotheca* increased throughout July to a peak in early August. It outnumbered all other species of algae combined throughout the season of 1946 and was present in large numbers in September 1939.

The zooplankton included the following species determined by Dr. S. Eddy of the University of Minnesota, with the exception of *Diaptomus* which was named by Mr. G. Comita, and certain rotifers which were determined by Dr. W. T. Edmondson, both of the University of Washington, Seattle.

Copepoda

Diaptomus arcticus Marsh.—A large and very abundant form.

Cyclops sp.—Scarce.

Cladocera

Alona costata Sars—Infrequent and only in shallow water.

Chydorus sphaericus (O. F. Müller)—Infrequent and only in shallow water.

Daphnia longispina (O. F. Müller)—Few, mostly inshore.

Eurycercus lamellatus (O. F. Müller)—Rare in plankton but abundant in dredgings and in the stomachs of trout.

Holopedium gibberum Zaddach—The dominant cladoceran of the open water.

Polyphemus pediculus (Linne)—Rare.

Rotifera

Brachionus variabilis Rousselet—Rare.

Collotheca libera (Zacharias)—Rare.

Conochilus unicornis Rousselet—Abundant and widespread.

Kellicottia longispina (Ehrenberg)—Abundant and widespread.

Monostyla sp.—Rare.

Polyarthra major?—Common in shallow water.

Protozoa

Ceratium hirudinella Müller—Common.

Difflugia sp.—Rare.

Peridinium sp.—Rare.

The zooplankton was dominated by *Diaptomus arcticus*, *Holopedium gibberum*, and the two rotifers *Conochilus unicornis* and *Kellicottia longispina*.

The copepod *Diaptomus arcticus* made up 30% of the numbers of zooplankters, and because of its extremely large size—more than 90% of the plankton volume. Preliminary tests of the movements of this species were made on August 19 and 20 at the deep water station (20 m.) in the south basin. Series of vertical hauls with a closing net were taken from 0–5, 5–10, 10–15, and 15–20 m. at four-hour intervals throughout a 24-hr. period. The results are indicated in the chart, Fig. 4. In the deep water, the greater

numbers of *D. arcticus* were taken at noon, suggesting some degree of downward migration at the period of intense sunlight. Large numbers were taken at the surface at midnight. However, the tremendous concentration at the surface in the evening, 8.30 p.m., must have involved horizontal as well as vertical concentration. Unfortunately, we were unable to follow up these interesting observations and have no explanations to suggest.

The cladoceran *Eurycercus lamellatus* was a second entomostracan of importance in the food cycle of the lake. While uncommon in the plankton hauls it was frequent in the dredgings and often abundant in the stomachs of trout. *Eurycercus* has been collected by the writer from numerous alpine lakes in the Rocky Mountains. Strøm (17) expresses the opinion that *Eurycercus glacialis*, found in Norwegian mountain lakes, is not a true relict but simply a cold water form of *E. lamellatus*.

Dry weight and ash determinations have been made on four total vertical hauls from July 3 to August 19. These are recorded in Table III. The average dry weight per haul was 11.75 mgm. The net used was calibrated against trap samples and shown to have a straining efficiency of 35%. Thus the catch of 11.75 mgm. in a net with a mouth 25 cm. in diameter is equivalent to 6.8 kgm. dry weight of plankton per hectare. This is the lowest standing crop of plankton reported from Canadian lakes, being even slightly lower than that reported by the writer for McLeod Bay (11). Although four samples cannot be regarded as adequate proof, they suggest that the plankton of Amethyst Lake is as low as would be expected in an extremely oligotrophic lake type.

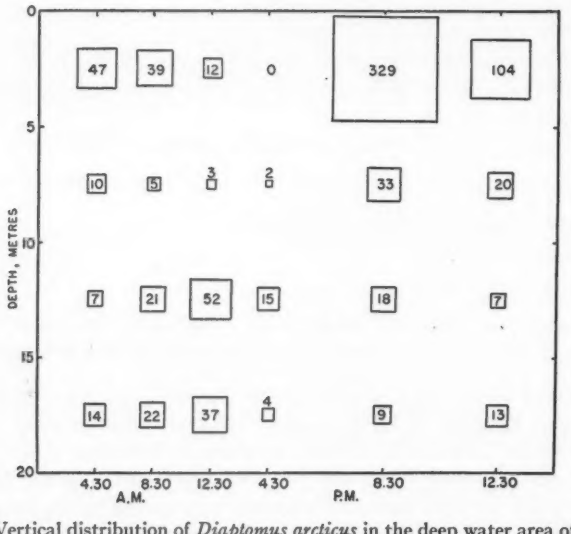

FIG. 4. Vertical distribution of *Diaptomus arcticus* in the deep water area of Amethyst Lake, August 19 and 20, 1946, as shown by 5-m. stage hauls taken at four-hour intervals through a period of 24 hr.

TABLE III

DRY WEIGHT AND ORGANIC MATTER IN FOUR TOTAL VERTICAL PLANKTON HAULS, AMETHYST LAKE, 1946

	Dry weight, mgm.	Organic matter, mgm.	Organic matter, %
July 3	17	9	53
July 19	10	6	60
Aug. 3	9	7	78
Aug. 19	11	9	82
Average	11.75	7.75	68.2

Bottom Fauna

The bottom fauna of the lake was explored by taking 70 samples with a small Ekman dredge. These were taken between July 17 and August 28 in 12 series which covered both basins. The dredge was 6 in. (15.2 cm.) square, thus bringing up an area of 1/43 of a square meter. The results of this sampling are summarized in Table IV which shows the composition of the bottom population and its depth distribution. The dredgings were supplemented with qualitative collections along the shores. In this area there is found a greater variety of aquatic insects and other invertebrates than that which was brought up by the dredging in the open water. It will be seen later, Table V, that these shore forms and the terrestrial insects are of considerable importance in the food of the trout.

The bottom organisms identified from Amethyst Lake are as follows:

Bryozoa

Fredericella sultana (Blum.) Several collections from various depths were identified as this species by Dr. Makato Toriumi, Tohoku University, Japan.

Turbellaria

A few planarians, possibly of the genus *Dugesia* were not suitably preserved for identification.

Nematoda

The nematode taken at various depths has been identified as *Hydramermis* sp. by Dr. L. J. Thomas, University of Illinois, Urbana.

Amphipoda

Gammarus limnaeus Smith. Common here and at even higher altitudes in lakes of the Banff National Park, south from Jasper.

Insecta

Plecoptera—Stonefly nymphs taken from rainbow trout stomachs on July 23 were identified by Dr. W. E. Ricker, Pacific Biological Station, Nanaimo, as *Isoperla fontium* and *Acroneuria pacifica*.

Ephemeroptera—Nymphs of the genus *Siphlonurus*.

TABLE IV

AVERAGE NUMBERS AND WEIGHT OF BOTTOM ORGANISMS PER SQUARE METER AT VARIOUS DEPTHS IN AMETHYST LAKE

Depth zones, m.	Number of dredgings	Sphaeriidae	Chironomid larvae	Cladocera	Oligochaetes	Amphipoda	Nematoda	Miscellaneous	Total numbers	Weight less mollusk shell, gm.
0.0– 2.5	11	1595	211	408	193	4.0	4.0	17.2	2432	5.07
2.5– 5.0	7	1130	99	378	155	6.0	12.1	15.9	1939	3.48
5.0– 7.5	14	1040	185	133	64.5	21.5	9.1	56.0	1509	1.93
7.5–10.0	17	972	464	113	94.5	73.2	20.2	22.4	1759	3.74
10.0–12.5	14	1175	640	58.5	94.8	40.0	6.0	18.5	2033	1.74
12.5–15.0	3	1550	460	43	159	—	14.2	14.2	2240	2.19
15.0–17.5	2	452	129	—	108	—	21.5	—	710	1.98
17.5–20.0	2	366	538	—	21.5	21.5	—	21.5	968	1.84
All depths	70	1035	341	142	111	20.8	10.9	38.6	1699	2.75
% Composition		60.9	20.2	8.3	6.5	1.2	0.6	2.3		

Trichoptera—Caddis larvae examined by Dr. H. H. Ross were found to represent *Dicosmoecus*, *Limnephilus*, and two other limnephilid genera.

Diptera—Chironomid larvae were abundant in dredgings from all depths. They have been identified by Dr. J. G. Rempel of the University of Saskatchewan, Saskatoon. *Chironomus* (*Stictochironomus*) sp. were most common in the shallow water. A few specimens of *Tanytarsus* were also found at middle depths. The Orthocladiinae were represented by *Spaniotoma* (*Psectrocladius*) sp., and the Tanypodinae by *Procladius* sp. *Spaniotoma* was abundant in the deeper water.

Gastropoda

Limnaeidae

Stagnicola catascopium (Say), identified by Dr. J. G. Oughton, Ontario Agricultural College, Guelph, was the only species collected. It was common in the shore collections and in the stomachs of trout.

Pelecypoda

Sphaeriidae

The sphaeriids identified by Mr. H. B. Herrington, Keene, Ont., include three species:

Pisidium conventis Clessin.—This was the dominant species at depths from 1 to 20 m.

Pisidium casertanum (Poli)—Few.

Pisidium subtruncatum Malm.—Rare.

The average number of bottom organisms per square meter is indicated in Table IV as 1699. This number was obtained by averaging the values for each of the 2.5 m. depth zones. By weighting the average according to the relative areas of the depth zones as indicated in Table I, a more representative figure of 1904 per sq. m. is obtained. Likewise, the average weight of organisms per square meter of 2.75 gm. may be corrected by weighting to become 3.17 gm. This is equivalent to 31.7 kgm. per ha. (or 28.2 lb./ac.). The approximate dry weight equivalent (15%) is 4.75 kgm./ha. This is a thin bottom population but slightly larger both in weight and numbers than that in most of the alpine lakes reported by the author (Rawson (9)) in 1942. Steinböck (15) found scanty plankton but bottom organisms up to 1680 per sq. m. in high alpine lakes of the Kühtai region. In a later paper Steinböck (16) reports an average of 770 organisms per sq. m. in the Schwarzsee, situated at an altitude of 2800 m. in the Austrian Tirol and free of ice for only two and one half to three months per year.

Trout

The introduction of rainbow trout, *Salmo gairdnerii*, into Amethyst Lake was described by the author in 1947 (10). In that paper reference was made to the rapid initial growth followed by deterioration and eventually by slow recovery. This sequence was observed in Amethyst and in five other lakes of the Canadian Rockies which had previously been barren of fish. The trout

of Amethyst Lake came from typical low altitude, Kamloops, stock and were planted in a very high alpine environment such as is commonly inhabited by the high mountain subspecies *Salmo gairdnerii whitehousei*. The trout were therefore observed closely to determine the nature of their adjustment to the new environment.

During the summer of 1946, gill nets from $1\frac{1}{2}$- to 4-in. stretched mesh were used to take 774 trout. A further 104 fish caught by anglers were also examined. The length–weight relationship for 700 fish was analyzed graphically and the following average weights were calculated. (The lengths given are standard—from the tip of the snout to the base of the caudal peduncle. These may be converted to fork length in inches by using a factor of 0.435.)

12 cm.— 28 gm.	25 cm.—215 gm.	40 cm.— 862 gm.
15 — 48	30 —374	45 —1190
20 —113	35 —585	48 —1400

The variation in weight in this sample of 700 rarely differed from the average shown by more than 10%. However, after spawning, fish more than 35 cm. in length frequently lost as much as 25% of their normal weight. With a few exceptions, the trout would be described as in good condition, in contrast to the situation observed in 1939 when all the older fish appeared to be emaciated. The condition of Amethyst Lake trout in 1946, as indicated by the relation of length to weight, was well within the range indicated by Mottley (6) for rainbow trout in Paul Lake.

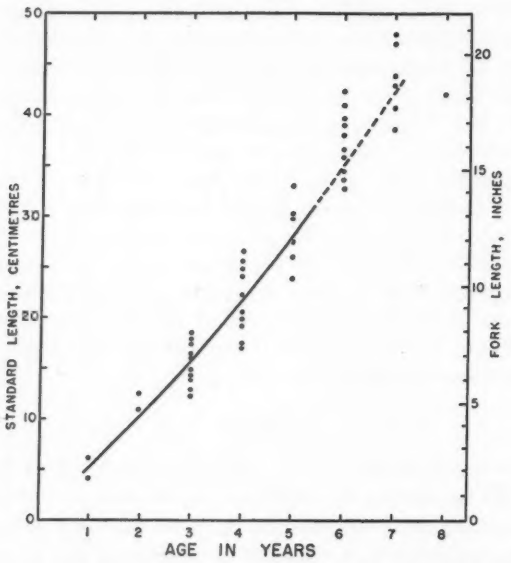

FIG. 5. Length and age of rainbow trout in Amethyst Lake, 1946.

Scales from 57 specimens have been examined for age determination and the resulting growth curve is shown in Fig. 5. This rate of growth is close to that reported for Pyramid Lake, Jasper (Rawson and Elsey (12)). It is slower by about 50% than that shown for the original stock of Kamloops trout, in Paul Lake, B.C. as reported by Mottley (6), Larkin *et al.* (4), and Smith (13). The present population of rainbow trout in Amethyst Lake are not particularly slow-growing in spite of the high, cold environment.

Spawning of the trout in Amethyst Lake was observed in the two inlet creeks and in the outlet immediately after the breakup of the ice in 1946. About 80% of the mature fish had spawned by July 15, but a few stragglers continued to spawn till mid-August. However, there appeared to be fewer adult fish with degenerating and partly resorbed eggs than had been observed in 1939. A small fraction of the adult population, mostly females, did not spawn and exhibited throughout the season the silvery appearance usually found in rainbow trout after the spawning season.

The food of the trout was examined in all the available specimens (about 875). The food of young trout in their first year appeared to be mainly cladocerans and especially the large *Eurycercus lamellatus*. After the first year, the small trout took the same food as the larger individuals although terrestrial insects were more frequent in the stomachs of the larger individuals. A distinct change in the diet of the trout was observed as the season progressed. This is demonstrated in Table V which shows the average stomach content of trout in three successive periods of approximately three weeks each. In the first period chironomid larvae are the main food with *Gammarus* and terrestrial insects. In the middle period *Gammarus* and sphaeriids are the main foods while in the third, entomostraca (mainly *Diaptomus* and *Eurycercus*) and terrestrial insects are dominant. Trout eggs were found in the stomachs of large fish of both sexes. These appeared to have been feeding at or in the streams, in which continuous spawning activity by many fish appeared to dig up eggs deposited by earlier spawning. No evidence of cannibalism was discovered although it is known among rainbow trout in other localities.

TABLE V

PERCENTAGE VOLUME OF FOODS TAKEN BY RAINBOW TROUT IN AMETHYST LAKE IN THREE SUCCESSIVE PERIODS

	June 29–July 16	July 17–August	8August 9–August 30
Chironomid larvae	50	22	+
Amphipods (*Gammarus*)	18	30	5
Terrestrial insects	12	12	20
Entomostraca (mostly *Eurycercus*)	—	8	60
Sphaeriids (*Pisidium*)	3	22	10
Caddis larvae	6	+	5
Gastropods (*Stagnicola*)	3	6	—
Trout eggs	8	+	—

Two intestinal parasites were found in the Amethyst Lake trout while the food of the trout was being investigated. These have been identified by Dr. R. R. Miller of the University of Alberta, Edmonton. The fluke *Crepidostomum farionis* (Müller) was fairly common. Plerocercoids of the tapeworm *Dibothriocephalus* were also found in a few trout.

It is evident that in 1946 the rainbow trout of Amethyst Lake were in good condition, spawning successfully and exhibiting normal rate of growth. This was not the case when the writer examined specimens from the lake in 1939. At that time the large fish were dark colored, emaciated, and many of them showed evidence of incomplete spawning. The fry for this stocking came from the Lloyds Creek Hatchery at Paul Lake, British Columbia, in 1931, 1933, and 1934. It is of interest to compare the stock in Amethyst Lake, 1946, with the original stock as seen in the Paul Lake environment.

The typical rainbow trout, *Salmo gairdnerii kamloops* of the interior plains of British Columbia are described by Dymond (1). The high mountain subspecies *Salmo gairdnerii whitehousei* (Dymond (2)), was distinguished by small size, several features of coloration including many dark spots and the retention of parr marks, larger numbers of scale rows, and fewer branchiostegals. The large fish observed by the writer in Amethyst Lake, 1939, were definitely of the original stock from the Lloyds Creek Hatchery. These, although in poor condition, had typical Kamloops coloration. The 1946 population had more dark spots than the typical Kamloops and many of them showed the orange tip on the dorsal, and white tips on the anal and ventral, characteristic of *S. g. whitehousei.* The males retained parr marks until they were 25 cm. in length and four years old. Since many of them spawned in their third and fourth years, this meant retaining their parr marks one or two years after spawning. However, the Amethyst trout were larger than the typical *whitehousei* which rarely exceed 12 in. (27 cm. standard length). Certain morphological characters suggest a condition intermediate between *kamloops* and *whitehousei.* The scale rows in 1946 ranged from 137 to 150 and averaged 146. In 1939, the larger fish, which were undoubtedly of the original stock, averaged 142 scales along the lateral line. However, the number of scale rows varies widely over the range of this species and our sample is much too small to warrant firm conclusion. The number of branchiostegals in 1946 was usually 11 with a few showing 10 and some 12, thus overlapping the range of both *kamloops* and *whitehousei.* Vertebral counts were made on 11 specimens which ranged from 62 to 65 vertebrae. Mottley (5) found no significant difference between the vertebral counts of the *kamloops* and *whitehousei* populations which he sampled.

Since the trout in Amethyst Lake spawn mostly in their fourth to sixth years, those examined in 1946 would be chiefly second and third generations. These fish appeared to be spawning more effectively than those originally planted in the lake. The color and conformation of the present stock suggest a shift toward the characteristics of the high mountain subspecies. However, there was no apparent dwarfing of the stock, and the available morphometric

data add only a little support to the above conclusion. As Dymond (1) points out, the subspecies was recognized as the extreme form in a series grading from typical *kamloops* to the high mountain type.

Amethyst Lake, at 1964 m., is certainly the highest lake in this region in which a satisfactory population of rainbow trout is maintaining itself. The cutthroat trout, *Salmo clarkii*, native to the east slope of the Rocky Mountains, thrives at somewhat higher altitudes in the Banff area, e.g. in Redoubt Lake, altitude 2300 m. (7650 ft.), east of Lake Louise.

Typology of Amethyst Lake

The significant ecological features of Amethyst Lake can be demonstrated by comparing it with three other alpine lakes of the Banff–Jasper region of the Rocky Mountains. The comparative data for these lakes have been selected from a previous paper (9) and they are presented in Table VI.

Amethyst and Bow Lakes are of similar size and altitude but Amethyst is clear and Bow is heavily silted. The mean depths of 7.3 in Amethyst and 17.6 in Bow are within the range which would ordinarily be associated with

TABLE VI

COMPARATIVE DATA FOR AMETHYST AND THREE OTHER LAKES IN THE BANFF–JASPER REGION OF THE ROCKY MOUNTAINS

	Amethyst	Bow	Maligne	Minnewanka
Altitude, meters	1965	1990	1663	1454
Lake area, km.²	4.7	3.6	21.8	13.0
Drainage area, km.²	26	75	775	170
Max. depth, m.	21	48	96	80
Mean depth, m.	7.3	17.6	40.5	38.1
Av. ice-free months	4.5	5.0	6.3	8.0
Temperatures, ° C.				
Maximum surface	12.1	11.0	13.3	15.4
Maximum bottom	5.7	7.4	4.1	6.3
Highest mean	9.9	8.3	6.2	10.8
Thermal stratification	Thermocline 10–13 m.	None	None	None
Minimum % saturation dissolved oxygen bottom	74	80	75	73
Transparency, Secchi disk, m.	4.5–11.0	0.4–1.5	0.5–2.0	8.0–11.5
Total mineral content, p.p.m.	—	85	104	198
Bottom organisms				
Av. number per m.²	1904	706	805	1141
Av. dry weight kgm./ha.	4.75	2.3	3.3	4.5
Average standing crop net plankton kgm./ha. dry weight	6.8	11.9	20.6	32.0

eutrophic conditions. It is clear from the small quantities of plankton and bottom fauna, that other factors outweigh the morphometric situation and the result is distinct oligotrophy. Amethyst is of particular interest in this regard. It has not only a eutrophic shaped basin, but has clear water and a thermocline. In spite of this, it has the lowest standing crop of plankton among the four lakes listed in Table VI. Presumably the low mineral content and the short season may be partly responsible for the poor plankton. On the other hand, the bottom population of Amethyst, although not rich, is heavier than that of the remaining three lakes. Probably its shallow depth and clear water favor growth of bottom plants which helps it to support a moderate bottom fauna. The shallow depth (and lesser volume) would increase the effect of rate of water replacement and thus tend to keep the mineral content low. Rapid water replacement has been suggested by several limnologists as a possible explanation for the low plankton production of high alpine lakes.

Amethyst Lake with its clear water and moderate bottom fauna supports a satisfactory population of rainbow trout. Bow Lake has a very limited fish production around its margin and is practically barren of fish in the open water. By comparison, it would seem probable that the heavy silting and scant bottom fauna were the main factors limiting the fish production in Bow Lake.

Maligne and Minnewanka Lakes are of comparable size and depth, but Maligne is silted and Minnewanka clear. Their mean depths of 40.5 and 38.1 indicate typical oligotrophic basins and the biological criteria show that they are oligotrophic. However, Minnewanka has clearer water, higher mineral content, and warmer water than Maligne and would therefore be expected to support more plankton and bottom fauna. Table VI shows plankton and bottom fauna in Minnewanka to be about one-third greater than that of Maligne.

The information now available on high alpine lakes of North America would not justify an attempt to classify them. European alpine lakes have been classified by Pesta (8) on a morphometric basis and by Steinböck (15) with emphasis on thermal conditions. Observations on Bow and Amethyst Lakes indicate that the trophic condition of a high alpine lake may be much more affected by climatic and edaphic than by morphometric conditions. In the extremely high lakes of the Indian Tibet, Hutchinson (3) found not oligotrophy but a degree of mesotrophy. He suggested that this may be the result of arid climate, which causes the nutrient salts to become concentrated, and possibly also the effect of intense solar radiation.

Conclusions

Amethyst Lake with an area of 4.7 km.^{2} and a maximum depth of 21 m., is located at an altitude of 1965 m. in the Rocky Mountains near Jasper, Alberta. It has a very small drainage area and only 38 p.p.m. total solids.

The ice cover persists from seven and one-half to eight months and the highest surface temperature is about 12° C. Thermal stratification is evident only in a small deep area. The water is relatively clear, well oxygenated, and slightly alkaline.

The plankton of Amethyst Lake is low in quantity (6.8 kgm. dry wt. per ha.) and especially poor in phytoplankters. The zooplankton is dominated by the large copepod *Diaptomus arcticus*. The bottom fauna averages about 1900 organisms per sq. m. and 31.7 kgm. per ha. It is dominated by the sphaeriids *Pisidium* (60%) and chironomid larvae, mostly *Stictochironomus* (20%).

Rainbow trout, introduced from 1931 to 1934, were of the typical *kamloops* stock from central British Columbia. After an initial period of deterioration they appear to have adjusted to the high alpine environment. In 1946, they were spawning effectively and maintaining a satisfactory growth rate. In some, but not all, respects they now resemble the high mountain subspecies *Salmo gairdnerii whitehousei*.

Amethyst Lake is alpine oligotrophic of a kind not previously described in North America. Its clear water and substantial bottom fauna are moderating influences in the extreme oligotrophy usually found in high alpine lakes. It is of interest that a good population of rainbow trout should maintain itself at this high altitude.

Acknowledgments

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VARIATION IN ANAL FIN RAY COUNT OF THE REDSIDE SHINER *RICHARDSONIUS BALTEATUS* (RICHARDSON)[1]

By C. C. Lindsey

Abstract

Anal fin rays were counted on 4766 specimens of *Richardsonius balteatus* from 61 localities in British Columbia. Individual counts varied from 10 to 21, and mean counts of different populations varied from 12.06 to 17.51. Significant differences in counts occurred between different bodies of water, between recently introduced populations and their parent stock, between different parts of the same lake, and between different year classes. Ray counts tended to be higher amongst females in populations with high over-all means, and higher amongst males in populations with low over-all means. A positive correlation was demonstrated between water temperatures recorded in the vicinity of developing fry and the mean numbers of anal rays produced. Within each latitudinal zone a similar correlation occurred between mean ray count and average air temperature during the spawning season, but data on 109 means of populations in U.S.A. and Canada indicated a tendency, probably genetic, towards production, at equivalent temperature, of higher ray count towards the northern end of the range. Loose correlations between anal ray count and certain other morphological characteristics suggest that these may be dependent on more or less common environmental factors but are not linked by direct causality.

Introduction

The tendency for many species of fish to have more meristic parts (such as vertebrae, scales, and fin rays) in the higher latitudes of their geographic ranges is well established. However, exceptions have been recorded (21), and these should be considered in the light of recent experimental studies on phenotypic variation (35) which suggest that no simple generalization can yet be made regarding the direct influence of temperature on meristic counts of fishes. In different species and in different series of the same species, the maximum number of parts may be produced by low, intermediate, or high temperatures. Other environmental factors may also influence meristic variation (35). It is therefore uncertain whether an observed latitudinal trend in meristic parts over the range of a species should be interpreted as variation produced by gradation in some environmental factor, or as a genetic cline, or as the interaction of both.

Intraspecific variation may readily be studied in the redside shiner *Richardsonius balteatus* (Richardson). This small cyprinid shows greater variability in number of anal fin rays than almost any other fresh-water fish which has been studied. Dense populations of shiners inhabit a wide variety of stream and lake types in the Pacific watershed of northwestern North America. Accordingly, as large samples can be obtained from a number of different habitats in each of several latitudinal zones, some distinction can be made between environmental and geographic variation.

The present study is based on field collections and observations made by the author in British Columbia in 1948, 1949, and 1950. Collections from the

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Royal Ontario Museum of Zoology and various other sources were also examined. Anal ray counts and other measurements were made on 4766 specimens from 61 localities. As a background to study of environmental variation, various phases of the life history of the species were also observed.

Literature on the Species

The redside shiner has been variously placed in the genera *Abramis* (*Cyprinus*), *Leuciscus*, and finally *Richardsonius*. Early nomenclature of the species is dealt with by Schultz and DeLacy (28). The common name has been given as Columbia River minnow, Richardson's minnow, silver-sided minnow, red sided bream, shiner, lake shiner, and redside shiner.

The known range of the genus *Richardsonius* lies west of the Rocky mountains between latitudes 56° and 39°. *R. b. balteatus* (Richardson) occurs in the coastal streams of Washington and Oregon, the Columbia River below Snake River Falls in Idaho, and the Skeena and Fraser systems of British Columbia. Recently a single specimen has been taken about 50 miles north of Prince George in a tributary to the Crooked River on the Arctic watershed (T. G. Northcote, personal communication). *R. b. hydrophlox* (Cope) occurs in the Columbia system above Snake River Falls and in the Bonneville Basin, and has been introduced recently into the Upper Colorado River (30). Subspecific distinction is based largely on anal fin ray counts. The only other species currently recognized (25) is *R. egregius* (Girard), occurring in the Lahontan system in Nevada and northern California (33), and having a lower anal fin ray count than *R. balteatus*.

In 1894 Eigenmann (11) published anal ray counts of *R. balteatus* taken from different localities on the Fraser and Columbia systems. While considerable variation occurred within single populations, there was also great variation between mean anal ray counts of different populations. He concluded that the number of rays in the species, and also the range of variation, decreased with increasing altitude (11, 12). However, anal ray counts subsequently published for other localities were in many cases contradictory to Eigenmann's generalization (13, 15, 17, 26, 29, 31, 32).

Distributional records until 1935 are listed by Schultz and DeLacy (28) and in other recent works (3, 5, 26, 30, 34, 36). Several earlier papers mention or illustrate scales of the species (7, 8, 9, 14). Measurements of specimens are given by Dymond (10) and Schultz and Schaefer (29). Food has been listed by a few authors (6, 27). Weisel and Newman (37) describe breeding and early development of the species in western Montana.

Occurrence of the Species in British Columbia

During the present investigation collections of shiners were examined from 61 localities in the Skeena, Fraser, and Columbia River drainages of British Columbia. These localities cover but do not extend the range of the species previously reported for the province in the literature; within this range shiners occur generally but not continuously. The species is apparently absent from

Teslin Lake at the northern boundary of British Columbia (4), and has not been reported authentically from Vancouver Island. The redside shiner shares its range with relatively few other species (63 fresh-water fishes are recorded from British Columbia as against about 200 from Ontario (3)). It is apparently one of the most successful of fresh-water fishes within its range, filling an important niche or series of niches in a number of habitats. It occurs in large lakes, moderately fast streams, and small ponds, with widely varying temperatures and trophic conditions. The species has probably invaded the northern part of its range from the south through the Columbia and Fraser systems following the retreat of the last glaciation (3). Its current absence from many lakes in British Columbia is probably due not to its inability to maintain its position there, but to its failure so far to gain entry because of geographic barriers. This contention is borne out by the explosive success of shiners introduced recently into such new localities as the Paul Lake chain near Kamloops and many lakes in the Kootenay district.

Life History

Temperature, depth, bottom type, and vegetation of waters inhabited by shiners vary greatly, and spawning behavior shows a corresponding flexibility. In 1949, the spawning period in southern British Columbia apparently varied from 7 to 10 weeks, starting in the last week of May in some localities and the second week of June in others. Cooler weather predominated in the spring of 1950, and in some localities spawning probably did not start until four weeks later than in 1949.

On July 5, 1950, shiners were observed to spawn in a small cold creek (9°–11° C.) a few feet above its entrance into Snowshoe Lake (west Kootenay), in a fashion similar to that described by Weisel and Newman (37). Males remained in a pool a short distance upstream; females ascended the stream from the lake after sunset. Spawning was evidently confined to the period from dusk to dawn; deposition of eggs was not observed after 0500 hours. During spawning a female and one or two males thrashed violently side by side for about two seconds. Eggs were deposited over fine gravel in one to three inches of rapid water between the pool and the lake. Eggs were adhesive when laid, and stuck to pebbles in the stream bed. Larvae when hatched were swept down into the lake.

In some localities shiners spawned over submerged inshore plants even though creeks were available. In Fleming Lake (Kamloops) on July 14, 1950, eggs were broadcast during the night or early morning over submerged masses of *Ceratophyllum*. The majority of these adhered to the leaves and developed within a foot of the surface, at temperatures of from 15° to 20° C. Eggs were also laid in other localities where there was no obvious movement of water. Unripe eggs were present at all times in most large individuals. Dissection of females from which ripe eggs had been stripped revealed quantities of unripe eggs. The majority of males provided free-flowing milt throughout the

spawning season. Possibly individuals may spawn several times in a season (as suggested by Weisel and Newman (37)), the production of eggs and sperms being a more or less continuous process.

Some shiner eggs were stripped and reared artificially in baths supplied by aerated water at controlled temperatures. The larvae so obtained died before developing anal fin rays, but the experiment yielded data on hatching time. Mean time from fertilization to hatching was 7 days at 21° C., 8 days at 18°, 11 days at 15°, and 15 days at 12°. (Weisel and Newman (37) found eggs at 21°–23° hatched in from 74 to 168 hours.) At 9° initial cleavage occurred, but 100 hours after fertilization development had ceased with the germinal disc appearing as a group of irregular cells scattered on the surface of the yolk. In these experiments, the parent fish were taken from water with temperatures of 15° or higher.

Early stages of development of the redside shiner are figured by Weisel and Newman (37), and follow in general the course outlined for cyprinids by Balinsky (2). Larvae hatch at a length (to the end of the notochord) of approximately 5 mm., and lie quiescent on the bottom for about a week. As yolk is assimilated, the young become more active, leaving the bottom to swim freely for increasing periods of time and beginning to feed. The first recognizable elements of the anal fin rays (as determined by examination of serial sections stained by Heidenhain's Azan technique) do not become visible in the mesenchyme until the larva attains a length of about 7.8 mm.

Growth rates of shiners differed considerably within the area studied. Where possible, ageing was determined from length-frequency distribution data, as scales were difficult to read. The mean standard lengths on September 1 varied, in different populations studied, from 17 to 27 mm. in the first year, from 34 to 55 mm. in the second, and from 55 to 75 mm. in the third. In all populations studied the majority of fish surviving beyond their third year were females. Scale examination of the largest specimen taken, from Shumway Lake (Kamloops), with standard length of 141 mm. (total length 170 mm.) suggested that it was probably in its sixth or seventh year.

The sex of mature fish may be determined by external appearance. Crimson and gold coloration of males is more vivid than that of females, particularly during the spawning season. Pectoral and pelvic fins are also longer in mature

TABLE I

STANDARD LENGTH OF SHINERS IN DIFFERENT DEPTH ZONES, ROSEBUD LAKE, AUGUST 28, 1949

Depth, ft.	Distance from shore, ft.	Number in sample	Percentage of sample in size range			
			10–24 mm.	25–39 mm.	40–59 mm.	60–80 mm.
1	6	16	100	0	0	0
2	12	63	6	29	62	3
4	20	58	0	36	55	9
9	30	79	0	3	83	14

males, the pectorals extending posterior to the origin of the pelvics in males but not in females. Local differences in anal ray counts of the sexes are discussed later.

Younger shiners show a marked tendency to stay closer inshore during daylight hours. Table I shows the size distribution of fish taken in different depth zones in Rosebud Lake (Kootenay) by means of a wire screen dip net drawn up at regular intervals. This segregation of different sizes of fish necessitates caution in obtaining samples for estimating size or age distributions. In addition, as sharp temperature differences over short distances occur, it is difficult to determine the precise temperatures to which fish are exposed during a stage at which they may be subject to environmental modification of structure.

Studies on movements of adult shiners in Kaslo Bay, Kootenay Lake, and in Paul Lake (Kamloops) were attempted by means of fin clipping. While the total numbers of fish proved to be too great for adequate population estimates, there was suggestion that shiners may frequent the same locality for considerable periods of time, and that semidiscrete populations may exist within a lake. This is in keeping with the difference in mean anal ray count of shiners from opposite ends of Paul Lake mentioned later.

Paul Lake probably contained between 5000 and 100,000 shiners per acre prior to the spawning period in 1950. Shiner populations of this density are not unusual in southern British Columbia and are obviously of considerable importance in the economy of the lakes they inhabit. Feeding habits of shiners are flexible. Items consumed include (roughly in order of importance) terrestrial insects, aquatic insect larvae, algae, mollusks, and young fish of their own and other species. Shiner fry subsist on diatoms, copepods, ostracods, and other small plankton organisms. Studies on the interaction of redside shiners and *Salmo gairdneri kamloops*, *S. clarki lewis*, and *Salvelinus fontinalis* have indicated that relative to these game species shiners may simultaneously assume the roles of predators, competitors, and prey.

Variation in Anal Ray Count

Anal fin ray counts were made with a binocular microscope. The last double ray was counted as one, and the two (rarely three) rudimentary rays anterior to the first long ray were omitted. Collections were examined from 61 localities in British Columbia, 30 lying between 49° and 50° N. latitude, and the remainder between 50° and 56°. Sample size was over 30 for 46 of the collections. Data on 4766 specimens are on file in the Department of Zoology, University of British Columbia.

Mean anal ray counts in British Columbia varied from $12.06 \pm .04$ to $17.51 \pm .15$. No obvious relation was apparent between ray count and altitude, latitude, habitat type, or drainage system. Populations separated by less than a mile differed markedly, while populations some 600 miles distant were similar. In general, populations with higher means displayed greater variability (relative as well as absolute).

Counts from three selected localities are shown in Fig. 1. These represent the lowest and highest means obtained (Erie Pothole, Kootenay and Nicola Lake, Kamloops) and one intermediate population (Pinantan Lake, Kamloops). Only one specimen from the collection with lowest mean lies within the range of the collection with highest mean.

The distributions for Pinantan and Nicola lakes display a striking phenomenon observed in several populations. In the former, males have a significantly higher anal ray count than females, while in the latter the reverse is true, the males having a significantly lower mean ($p < 0.01$). These sex differences are present within single year classes, and are not, therefore, attributable to differential mortality in successive year classes having different means.

Females had significantly more anal rays at Queen's Bay, Kootenay Lake (p 0.02–0.05), while males had significantly more rays in Snowshoe Lake (p 0.01–0.02) and in the west end of Paul Lake (p 0.02–0.05). The remaining 14 collections in which the phenomenon was investigated did not show differences which were statistically significant.

However, considering only populations studied for this phenomenon in which over 28 specimens of each sex were examined, the six populations having over-all means less than 15 were contrasted with the five populations having over-all means greater than 15. In the former the mean female count was 0.24 less than that of the males, while in the latter the mean female count was 0.26 greater than that of the males (p 0.02–0.05). In general, then, there appeared to be a trend towards relatively higher female ray counts in populations having high over-all means, and towards relatively higher male counts in populations with low over-all means.

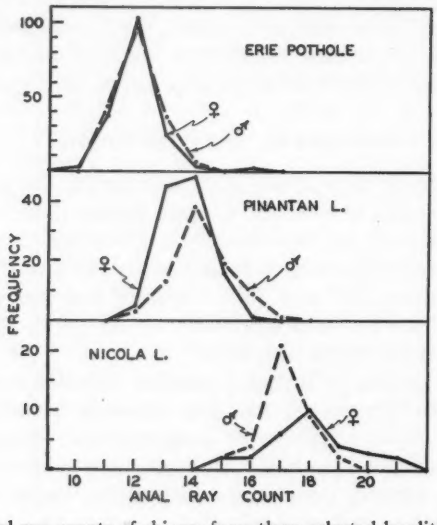

FIG. 1. Anal ray counts of shiners from three selected localities.

Evidence for Environmental Control

Various lines of evidence indicate that observed differences in anal ray count are at least partially the result of environmental control.

In the Paul Lake chain of lakes near Kamloops, shiners were reportedly first introduced some years ago into Hyas Lake near the top of the chain. They spread into Pinantan Lake sometime after 1930, and from there entered Paul Lake about 1945. The mean ray count of a sample from Pinantan in 1948 was significantly lower than one from Hyas (p 0.02–0.05). Ray counts of fish from the east end of Paul Lake near the creek from Pinantan Lake did not differ significantly from Pinantan counts, but counts of fish from the west end of Paul (three miles distant) were significantly lower ($p < 0.01$) than those from the east end and those from Pinantan.

Mean anal ray count may also vary from year to year at one locality. Mean count of shiners of all sizes collected from Pinantan Lake in 1946 was significantly lower ($p < 0.01$) than the mean of individuals taken from the same place in 1948. The higher mean of the 1948 sample appeared to be due largely to one-year-old fish which showed a higher mean than older individuals. (Despite annual variation found in some populations, ray counts of shiners taken by the writer at Sicamous on Shuswap Lake in 1949 did not differ significantly from counts reported for the same locality by Eigenmann in 1895 (11)).

Variation in ray count also occurs within single year classes. In most samples, the larger fry had somewhat higher ray counts, although the reverse trend (not statistically significant) was found in a few. As an example of the former, Pinantan Lake fry taken on August 18, 1948, showed a significant positive correlation between standard length and anal ray count (p 0.01–0.02).

In summary, significant differences in anal ray counts have been found between fish in different bodies of water, between recently introduced populations and their parent stock, between fish in different parts of the same lake, and between collections made in different years from the same locality. Within a year class, the larger fish sometimes have significantly more rays, and possibly they sometimes have fewer. There seems little doubt that in this species, environment exerts a considerable influence on the number of anal rays. In addition, sex differences in ray count exist which may also be subject to environmental modification.

Correlation with Temperature

Of possible environmental factors governing ray count, temperature appears to be the most obvious. Rearing experiments at controlled temperatures were attempted, but failed owing to the death of all fry (probably as a result of improper feeding) before anal rays were visible. It was therefore attempted to record temperatures to which wild fry of various populations were subject.

Maximum–minimum recording thermometers were suspended in localities frequented by newly emerged larvae. As sharp temperature gradients,

diurnal changes, and movements of the larvae prevented precise recording of temperatures to which larvae were subject, these observations indicate only probable temperature limits within which anal rays were developed.

Fig. 2 shows the means and ranges of anal ray counts of adult shiners from 11 localities, plotted against the observed temperature medians and ranges to which larvae were probably subject. In all cases, eggs or young fry were actually collected in the vicinity where the temperatures were recorded. Broken lines indicate a possible extension of the lower temperature limit in localities where night temperatures were not recorded.

Considering the limits of accuracy of the observations, there is a strong positive correlation between anal ray count and developmental temperature. No such correlation was evident between ray count and dissolved materials, lake type, or obvious biotic factors. Evidently, temperature is an important factor in controlling the number of anal rays in the species.

Some of the variation in ray count observed within, as well as between populations, may also be attributable to temperature. Rising water temperatures throughout a prolonged spawning season would tend to produce lower ray counts among the larger fry (which had been subject to lower temperatures during the fixation period); falling water temperatures would produce the reverse trend. However, ray count might also be related to the growth rate of individual fish. Heuts (19) showed that, within a single brood of *Gasterosteus aculeatus* raised under constant conditions, the faster growing individuals had a greater number of lateral plates. Similarly, the writer has found a positive correlation between dorsal and anal ray and basal counts and size within artificially reared broods of the same species (unpublished). Gosline (18) found in a wild population of *Poecilichthys exilis* two sets of correlations, one involving standard length, scales, vertebrae, and dorsal spines, and another independent correlation between numbers of dorsal and anal rays. In *R. balteatus* an association between ray count and growth rate might tend to obscure size–ray count trends produced by changes in water temperature during the spawning season.

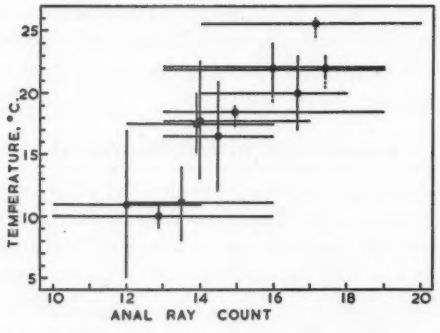

FIG. 2. Relation of mean and range of anal ray count to median and range of observed water temperatures during development.

Intrapopulation differences in ray count must also be considered relative to the sexes. Evidence has been presented suggesting that higher ray counts occur among the males in populations with low mean counts, and among the females in populations with high means. This may be the result of greater susceptibility of females to temperature control. (Gabriel (16) showed genetic differences in *Fundulus* as to the degree of lability of vertebral count under different temperatures.) Alternative speculations might be advanced, but it is clear that further knowledge is required of the degree of genetic variation within single populations.

Local differences due to the action of sunlight, wind, current, or springs may affect water temperature profoundly. Nevertheless, if temperature affects ray count, a correlation between mean air temperature during the developmental period and ray count should be apparent when a sufficient number of localites from a number of different air temperature zones are considered. Furthermore, the pattern of mountain trenches in the range of *Richardsonius* results in a general north–south rather than east–west orientation of temperature isotherms; hence ray counts at different latitudes may be compared within the same temperature zone.

Table II summarizes mean anal ray counts at 51 localities in the United States from which ray counts have been recorded, and 58 localities in British Columbia examined by the writer. Means for individual localities have been given equal weight. United States localities are grouped according to the average summer air temperature isotherms between June and August as given in the Atlas of American Agriculture (1) (based on a 20-year average of means of daily extremes). No comparable isotherm maps were available for British Columbia; mean temperatures for the same months were approximated from Provincial Department of Agriculture records or from observations of actual water temperatures.

TABLE II

MEAN ANAL RAY COUNTS WITHIN AIR TEMPERATURE ISOTHERMS
(Number of localities in brackets)

	Latitude, °N.	Average summer temperature, °C., June-August				
		7–12°	12–15°	15–18°	18–21°	21–24°
R. b. balteatus Canada	49–55	12.83 (4)	14.69 (8)	15.53 (33)	15.77 (11)	17.24 (2)
R. b. balteatus United States	42–49		13.10 (5)	14.30 (23)	16.27 (3)	16.80 (8)
R. b. hydrophlox	41–43			11.63 (9)	13.40 (3)	
Combined mean *R. balteatus*		12.83	14.08	14.55	15.44	16.89
R. egregius	39–42			8.40 (1)	8.54 (3)	

In Table II mean ray counts tend to increase from left to right and from bottom to top. Thus a positive correlation between air temperature and mean anal ray count appears to exist in each latitudinal zone; moreover, within a given temperature zone there is, with one exception, an increase in mean ray count from south to north. These latitudinal differences in the apparent effect of temperature might be attributable to some environmentally controlled factor such as difference in day length or radiation intensity. Alternatively, a genetic cline may be involved. The latter hypothesis is supported by the striking difference in ray count of *R. egregius* shown on the bottom line of Table II, occurring immediately to the south but having markedly lower mean ray count than *R. b. hydrophlox* in equivalent temperature zones (22, 23, 33). Such a difference is almost certainly too great to be attributable to environmental changes dependent on the slight difference in latitude, although the possibility of sharp geographic differences in such factors as dissolved solids cannot be ruled out.

Possibly, while ray count is partially determined by environment, the range of variation over which environment may operate is genetically fixed for each area, but differs between different areas. If so, a genetic tendency toward higher ray count from south to north is masked by an environmentally controlled tendency toward higher ray counts at higher temperature.

Eigenmann (12) claimed a negative correlation between altitude and anal ray count, as discussed previously. Probably such a general relation does exist in so far as higher altitude is associated with lower temperature, but such a relation is largely masked in the parts of the Columbia system from which opponents of Eigenmann's generalization drew their examples. Sections of the Columbia and Snake rivers falling within the hottest summer isotherms (in the vicinity of Walla Walla, Wash., and again in the vicinity of Nampa, Ida.) are at a higher elevation than cooler coastal areas. Hence ray count, which probably follows temperature, does not inversely follow altitude in these areas.

Correlation with Other Structural Variation

Shiners show considerable variation in characteristics other than anal fin ray count. The degrees of correlation were investigated between anal ray count and vertebral count, scale count, and certain body proportions.

Vertebral counts were made from X-ray plates of 109 shiners with anal ray counts from 11 to 20. Counts started at the first vertebra bearing a neural spine, and included the hypural plate. Mean count was $37.16 \pm .06$ with a range from 36 to 39. While scatter was considerable there was a tendency for higher ray count to be associated with higher vertebral count. Mean vertebral count for fish with from 11 to 15 rays was significantly lower than the mean for fish with 16 to 20 rays ($p < 0.01$). In order to obtain a sufficiently wide range of variation in rays, fish from different populations were included in these counts, so that apparent correlations must be treated with caution.

No significant difference was found between vertebral counts of the sexes. Similarly the numbers of vertebrae posterior to the first proximal radial of the anal fin were similar in the two sexes (range 18 to 21, mean 19.81 ± .08).

Counts of lateral line scales were made on 115 fish from Snowshoe Lake and Inonoaklin River (west Kootenay). These varied from 54 to 67, with a mean of 60.98 ± .25. As in the case of vertebral count, scale count showed a loose positive correlation with anal ray count within the two populations studied. Mean ray count of Snowshoe Lake fish with 54 to 61 scales was significantly lower (p 0.02–0.05) than those with 62 to 67 scales.

Regarding variation in body proportions of fish, Martin (24) has suggested a mechanism for environmental control. A plot of body length against body part on log–log axes shows that early growth of many parts of fish is characterized by a series of "stanzas", each with a different growth constant relative to the standard length of the fish. Transition from one stanza to the next is usually abrupt. Parts growing slower than the whole are said to show bradyauxesis, parts growing faster show tachyauxesis. According to Martin, successive growth constants are probably alike for all individuals of a species, but body size at inflection from one stanza to another is subject to environmental control. Factors such as food or temperature causing inflection at a smaller body size result in a relatively larger or smaller body part depending upon the direction of inflection. The sharper the inflection the greater the diversity likely to result.

Relative growth of several body parts of shiners was plotted. Length from snout to anus ("preanal length") was used rather than standard length, because of the inflections in growth of the caudal region. As might be predicted from Martin's hypothesis, those parts displaying the sharpest inflections in early growth also showed the greatest variability in adult proportions. Eye diameter and head length showed sharp inflections from tachyauxesis to bradyauxesis at a preanal length of about 11.0 mm. Correspondingly, eye diameters and head lengths of adults with preanal length of 30.0 mm. showed wide variation, from 3.5 to 4.9 mm. and from 10.1 to 14.0 mm. respectively.

Variation in fin length between the sexes is also due to differences in point of inflection between growth stanzas. The longer pectoral and pelvic fins of males in all populations result from a period of accelerated relative growth of these parts which commences at a smaller body size in males than in females.

Postanal length (measured from the origin of the anal fin to the tip of the caudal peduncle) shows a more complex type of growth. The tail bud of the embryo starts to grow when the total length of the unhatched embryo, curved around the yolk sac, is approximately 3.2 mm. The tail region grows rapidly until it is approximately 2.0 mm. long, during which time the preanal length has increased to approximately 3.6 mm. There is a sharp inflection at this size, postanal growth showing bradyauxesis after hatching. Although there is some indication of later and less pronounced inflections, it is probably the sharp early inflection which is largely responsible for the considerable adult variation in postanal length.

Anal ray count showed a stronger correlation with postanal length than with other body proportions studied. In order to compare postanal length of fish of different sizes it was necessary to correct for the differential growth of the two portions of the body, as the preanal portion grows faster than the postanal throughout adult life. Each postanal length was therefore converted to the corresponding measurement at an arbitrarily chosen preanal length (30 mm.) by assuming that the slope of the line of best fit for the log–log plot of postanal against preanal length of all fish also described the growth of each individual (24). When this was done, a distinct trend was evident towards greater postanal length in individuals with higher ray count. However, the maximum difference in postanal length (between fish with 12 and those with 20 rays) was only about 16%, so that variation in ray count cannot be attributable directly to variation in length of the postanal part of the body to which it is attached. Moreover, correlation between individual ray count and postanal length is not precise enough to suggest that there is direct causality between the two.

As can be shown by measurements of the lengths of fin bases of fishes with varying numbers of anal rays, fins with more rays occupy a greater proportion of the postanal region. This is apparent even by superficial examination of fish having high and low ray counts, the fins of the former extending much farther posteriorly. The relationship is partly concealed in a sample of fishes of varying sizes by the fact that the proportion of the postanal region occupied by the anal fin base continues to increase slightly between standard lengths of 20 and 100 mm. Apparently, during adult growth, the fin base elongates more rapidly than the ventral surface of the peduncle posterior to it. However, the proportion of the postanal length occupied by the bases of fins with high and low ray counts bear approximately the same relation to each other in fish of both 20 and 100 mm. standard length.

While fins with more rays have longer bases, the relation between fin size and ray count is not a strict proportionality. Fish with high ray count have longer fin bases but also have their rays closer together; this apparently applies to the spacing of all rays in the fin and not merely to the more posterior ones. The number of proximal radial elements is not a constant ratio of the number of somites they occupy (nor, therefore, of the number of haemal spines in the region of the anal fin), nor of the number of spinal nerves supplying the anal fin muscles. Also, the number of fin rays does not correspond exactly to the number of proximal radial elements supporting them, nor to any simple fraction thereof; there is a variable degree of fusion of proximal elements, especially at the anterior and posterior ends of the fish. Evidently the spacing and extent of the anal fin ray series is largely autonomous.

A rough positive correlation was found between eye diameter and the number of anal rays. Champion Lake shiners, with high ray counts, had greater eye diameter ($p < 0.01$) than Erie Pothole and Paul Lake fish which had lower ray counts; other populations studied were intermediate in eye diameter and ray count.

Other body proportions studied did not show a close correlation with anal ray count. One reason may perhaps be found in the difference in the times at which these characters are determined. Rays first become visible (in stained sections) at a standard length of about 7.8 mm., and their number may well be fixed at a somewhat earlier stage. On the other hand, inflections in most body parts occur a considerable time after the anal rays are determined, by which time the fish has probably moved to a somewhat different environment and has also become subject to variations in diet. It is not surprising that variations in a character probably determined while the embryo lies comparatively immobile and subsists on its yolk reserves are not closely linked with variations of characters probably finally determined in the later pelagic and feeding phase.

Ray count has been shown to bear a closer relation to postanal length than to other characters studied, and here the major inflection occurs at an earlier stage (5.6 mm. standard length as contrasted to 11.0 mm. for eye diameter, head, etc.). Presumably determination of postanal length occurs closer to the time of ray count determination, and environmental change between the two events is less likely to be pronounced.

Determination of vertebral and scale count probably occurs before inflections in growth of body parts described, and both characters show a loose positive correlation with ray count. The factors controlling these two characters are unknown for the species, but the positive correlation between temperature and ray count might suggest some association between high temperature and high scale and vertebral count.

Discussion

The positive correlation which has been shown between observed developmental temperature and the number of fin rays produced suggests the existence of pronounced phenotypic plasticity, although in the absence of controlled rearing experiments the possibility of differential mortality at different temperatures cannot be ruled out. There is also suggestion of a genetic cline which affects the range over which temperature can modify anal ray count. This may be comparable to cases cited by Hubbs (20), who suggests that graded series of races of fish varying in number of body parts are produced by selection acting not directly in favor of adults with more or fewer parts, but rather in favor of certain developmental velocities which incidentally result in more or fewer parts. At any rate, geographic variation of the present species, though largely obscured by phenotypic variation, probably follows the classical pattern with more parts produced (under equivalent environmental conditions) towards the northern end of the range.

The relationship between serial parts of the median fins and the segmentation of the axial skeleton of fishes is not clear; in some species there appears to be a fixed relation between basal fin ray elements and vertebrae, while in others no strict serial arrangement is evident. It is uncertain whether variation in the number of parts in a meristic series is due to differences in the

dimensions of the presumptive tissue at time of segmentation, or to environmental influence on internal spacing of the series, or to some combination of these or other components. Varying directions and degrees of influence of these factors could produce the apparently contradictory results of temperature on segmentation reported for different species.

In *R. balteatus*, variation in the anal fin has been shown to be largely independent of vertebral variation. The rather loose correlations between anal ray count and the other variable characters which have been discussed, suggest that ray count and these characters are perhaps dependent upon more or less common environmental factors, but are not linked by any direct causality.

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THE SALIVARY GLAND CHROMOSOMES OF THE BLACK FLY *SIMULIUM VITTATUM* ZETT.[1]

By K. H. Rothfels[2] and R. W. Dunbar[3]

Abstract

Neuroblast and salivary gland chromosomes were studied in Feulgen squash preparations of *Simulium vittatum* larvae. The haploid complement consists of three metacentric chromosomes; the arm ratio of the longest (1) and shortest (3) is approximately as 1 : 1, that of the second as 1 : 2. Centromere regions of salivary gland chromosomes are expressed as characteristic expanded drums with large disorganized chromomeres. The third salivary gland chromosome bears the single large nucleolus and a smaller granular structure comparable to the Ring of Balbiani of chironomids. Pairing of the two constituents of salivary gland chromosomes is quite loose regardless of the degree of polyteny. Photographic maps are given of the standard band sequence of the entire complement. Relatively short inversions are the commonest type of structural rearrangement. From preliminary observations on further black fly species and an eclectic treatment of the nematocerous literature, morphological characteristics of salivary gland chromosomes are discussed with respect to their value in delimiting taxonomic segregates and determining phylogenetic relationships.

Introduction

During the last year we have begun a cytological survey of the black flies of eastern Canada. A preliminary study has been made of some 20 species.

The Simuliidae are of interest to entomologists and cytologists alike; to the former largely because of their practical significance as pests and disease carriers, to the latter because of their exceptionally large and "workable" salivary gland chromosomes. These were first studied by Geitler (17) in an unnamed European form and by Painter and Griffen (23) in *S. virgatum*.

The black flies form a closely knit group with some 70 species already described from Canada and probably few remaining undiscovered. About one half of these species are western. Taxonomy of the imago and, in a measure, of the pupa is well advanced but "larval keys" are lacking, necessitating arduous rearing for the identification of immature stages. Information on the biology and taxonomy of species occurring in Canada is to be found in the papers of Twinn (34), Twinn *et al.* (35), Davies (10, 11), Hocking and Richards (19), Shewell (29), and Puri (27, 28)*.

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Contribution from the Department of Botany, University of Toronto, Toronto, Ont. This work was initiated in the summer of 1951 while the junior author was engaged as an assistant under a grant from the Defence Research Board to Dr. F. P. Ide. Continuation was made possible in 1952 through a grant from the Research Council of Ontario.

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* *For the purposes of the present paper, technically correct naming of black fly species other than* vittatum *is not of primary importance. In general we have followed the usage embodied in the recent writings of Davies (10, 11), Shewell (29), and Stone (33). Citation of authors has been omitted except where determinations are critical. Reference is made below to three controversial points:*

(*1*) *We have accepted the synonymy of* Cnephia lasciva (*Twinn*) *with* C. dacotense (*D. & S.*). *material of this affinity from the type locality of* lasciva *is referred to as* dacotense.

(*2*) *We have accepted the synonymy of* Simulium perissum *D. & S. with* S. tuberosum (*Lund.*).

(*3*) Eusimulium *in this paper is given generic rank to avoid extensive use of trinomials.*

These arbitrary stop-gap procedures should not prejudge situations the resolution of which is outside the special competence of the writers.

A general review of the family was given by Smart in 1945 (30). Native forms are now classed into five taxonomic segregates: *Gymnopais*, *Prosimulium*, *Cnephia*, *Eusimulium*, and *Simulium* s. str. The genus *Gymnopais* erected by Stone (32) on two arctic species, and including as yet undescribed forms from New York State and Labrador, stands clearly apart. Similarly the *Prosimulium* appear to form a "natural group" although the delimitation against *Cnephia* is not everywhere clear. The species assemblages at present included in *Cnephia*, *Eusimulium*, and *Simulium* are probably polyphyletic. This is reflected both in shifting generic placement of species such as *subexcisum* Edwards and in disagreement as to rank and definition of proposed segregates. Further studies of immature stages are apt to multiply rather than resolve these difficulties as indicated for example in Stone's (33) attempt to provide a generic description of *Cnephia* pupae. Controversies become even more pointed when faunae other than nearctic are brought into the argument (22).

Chromosome studies have resulted in a successful elucidation of phylogenetic relations in *Drosophila* as summarized by White (36), and in the Chironomidae as studied by Bauer (2, 3). Similar studies, based primarily on salivary gland chromosomes, were begun in sciarids by Metz and students, in cecidomyids by White (37, 38), in *Anopheles* by Frizzi (15, 16), and in *Culex* by Kitzmiller (20). It is obviously tempting and promising to apply this approach to the Simuliidae.

In black flies the giant salivary gland chromosomes provide a wealth of descriptive morphological detail in their number and gross morphology, in the characteristics of expanded centromere regions, in the location of specific nucleolar sites, in the degree of pairing of constituents, and in the ultimate discernable banding pattern. These last characteristics in particular are as close to the primary genetic structure as one can get, they are recognizable in larvae and pupae and may be used to define segregates. Furthermore, since these features are not functionally related to the external environment the confusing effects of convergence are minimized, resemblances may be taken to indicate relation, and grouping is possible according to natural affinities. Beyond this, comparison of the banding pattern of related segregates may reveal the structural (as opposed to the gene-mutational) correlates of evolutionary diversification such as interchanges and inversions, especially where these form overlapping series. Finally in certain instances the direction of evolutionary processes may be inferred as where the chromosome number is reduced by rearrangements involving centric fusion (*Eusimulium euryadminiculum:* $n = 2$), or where it is increased by changes in ploidy (*Cnephia mutata:* $3n$).

The present paper gives a detailed description of the chromosome situation in *Simulium vittatum*. This is the preferred test material since, at least in Ontario, it is widely available at all times and yields good preparations more readily than other species. It is hoped to describe the cytology of other species groups against this background.

Materials

Larvae of *S. vittatum* may be found in appropriate localities at all times of the year attached to rocks, submerged weeds, and debris. They are less sensitive to pollution, high temperatures, and sluggish current than most other forms. The species is very widely distributed, practically cosmopolitan, and occurs in North America from coast to coast and from Mexico to the Arctic. Descriptions of all stages and data on life history and biology were given by Twinn (34).

Mature larvae with fully developed pupal respiratory histoblasts yield the best salivary gland chromosomes as well as neuroblast metaphases and spermatocyte stages, but younger larva and early pupae may be used. Dissection of the 16-branched pupal respiratory organs confirms identification and younger larvae may be recognized by the characteristic head pattern, tooth details of the submentum, and extent of ventral incisure of head capsule. The sexes may be distinguished both in living larvae and in Feulgen-stained bulk material since ovaries are larger than testes as first shown by Puri (27) for *S. decorum* (*nölleri*).

During the winter in the Don river at Toronto, last instar larvae were not found but penultimate instar larvae were common and were readily brought to pupation when kept in cool bubbled water. From early April till late in the fall mature larvae could always be collected in certain creeks near Toronto, although not all bodies of water yielded mature larvae simultaneously owing to differences in water temperatures, rate of flow, and other factors. There are apparently several generations a year; a clear-cut diapause is lacking.

Slides were prepared primarily from collections taken at various localities within a 30-mile radius of Toronto, others from larvae collected near Collingwood, Ont., and a considerable contingent from Fort Churchill, Man. Larvae were either picked with forceps off rocks and weeds or were shaken off aquatic vegetation and caught in a 16-mesh-to-the-inch sieve. When stored in a refrigerator on moistened filter paper they would keep for about a week.

Cytological Methods

After testing various staining methods, especially those involving aceto-carmine or acetic-orcein, with and without prefixation and hydrolysis, three types of mounts were settled on for routine examination of this material. These were: (1) living mounts of entire glands in "Nujol"; (2) Carnoy–Feulgen–euparal permanent mounts for band mapping and for neuroblast and spermatocyte chromosomes; (3) chrom formal – Feulgen – fast green mounts for study of nucleolar relationships.

The salivary glands of the simuliids are large, traversing the whole length of the body and doubling forward so that the blind end is near the center of the body (for figures see Puri (27)). The largest and most useful nuclei are in the posterior third of the larva. Glands were exposed, wholly or in part, by tearing the body wall with sharp needles in a very small drop of water.

For living mounts the glands, dissected in the body fluid, were covered with "Nujol" and a coverslip. Such mounts were especially useful for determining number and size of nucleoli. Phase optics are not required. Bands can be seen clearly but since the chromosomes are much longer than those of the chironomids it is generally not possible to trace them end to end without rupturing the nuclear membrane.

For stained preparations larvae were fixed, no more than 12 per vial, in either Carnoy (3 : 1 or 2 : 1) or a freshly prepared Levitsky type of mixture (equal parts 1% chromic acid and 4% formaldehyde). Fixation in acetic alcohol was for five minutes to 24 hr., in Levitsky for one hour. Glands and, if desired, oesophageal ganglia and imaginal disks were rapidly exposed and the whole cadaver was fixed to serve as a handle by which to transfer organs through a succession of reagents. Speed in dissection is essential. Levitsky fixation was followed by washing in running water for one hour to remove aldehyde. Material fixed in Carnoy did not require washing. Hydrolysis followed in *N* hydrochloric acid at 60° C. for four to seven minutes after Carnoy and for 10 to 15 min. after Levitsky. Feulgen reagent was prepared as described by Coleman (9). Staining was for 15 min. to two hours and was followed by two changes of sulphur dioxide water of three minutes each. Stained Carnoy glands were dissected under a binocular microscope and individually squashed in 50% acetic acid between slide and coverslip until a desirable degree of spreading was reached. Such mounts were either studied immediately, or ringed with vaseline–beeswax mixture and stored in the refrigerator for a few days, or made permanent as described below. Levitsky material was counterstained in bulk with 0.2% aqueous fast green for ca. 15 min., rinsed in 50% acetic acid, and dissected and squashed like Carnoy material.

Preparations were made permanent by immersing temporary mounts face up in a large Petri dish of 95% alcohol, separating cover and slide after 12 hr. or more, and reuniting with euparal brought to the proper consistency with absolute alcohol. This procedure is less troublesome than dioxane, vapor replacement, or freezing methods and the results are in no way inferior. Chromosomes do not shrink and show no signs of fading after more than a year of keeping.

For measurements of salivary gland chromosomes, flattened and evenly stretched specimens were outlined with a camera lucida and traced with malleable copper wire which was then straightened and measured on a stage micrometer scale reproduced under the same optical system.

Slides were examined and photographed through Leitz Fluorite or 90× apochromatic objectives N.A. 1.3 and oil immersed achromatic condenser N.A. 1.4 with a compensating 12× eyepiece. Illumination was either from a Hamly H lamp or from a Bausch and Lomb ribbon filament with a Farrand interference filter of transmitted peak wave length of 575 mμ.

Photography was done with a Leica 35 mm. fitted with ipso adapter using Dupont's panchromatic microcopy (DK 20) for ganglia and lightly stained salivaries, and using Ilford panchromatic microneg (Id 48) for more heavily stained chromosomes. Prints were made on Kodabromide F_2 to F_4.

Results and Discussion

I. Chromosome Number and Morphology

Mitotic metaphases from neuroblasts or imaginal disks of *S. vittatum* regularly show three pairs of metacentric chromosomes somatically paired (Fig. II). One pair (No. 1) is clearly longer than the other two and its primary constriction or centromere is very close to the center. The two shorter chromosomes are of very nearly equal over-all length but can be distinguished by the position of the centromere which in one (No. 2) is in the one-third position while in the other (No. 3) it is practically median. The largest chromosome may measure up to 6 μ in length in full metaphase. Preparations were not sufficiently favorable to determine the attachment point of the nucleolus in ganglion chromosomes. Interphase nuclei show no conspicuous chromocenters (Fig. II).

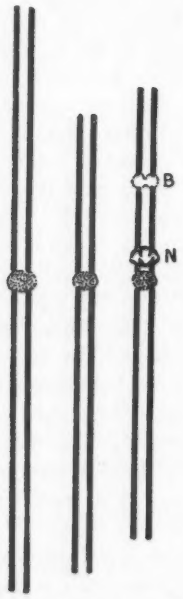
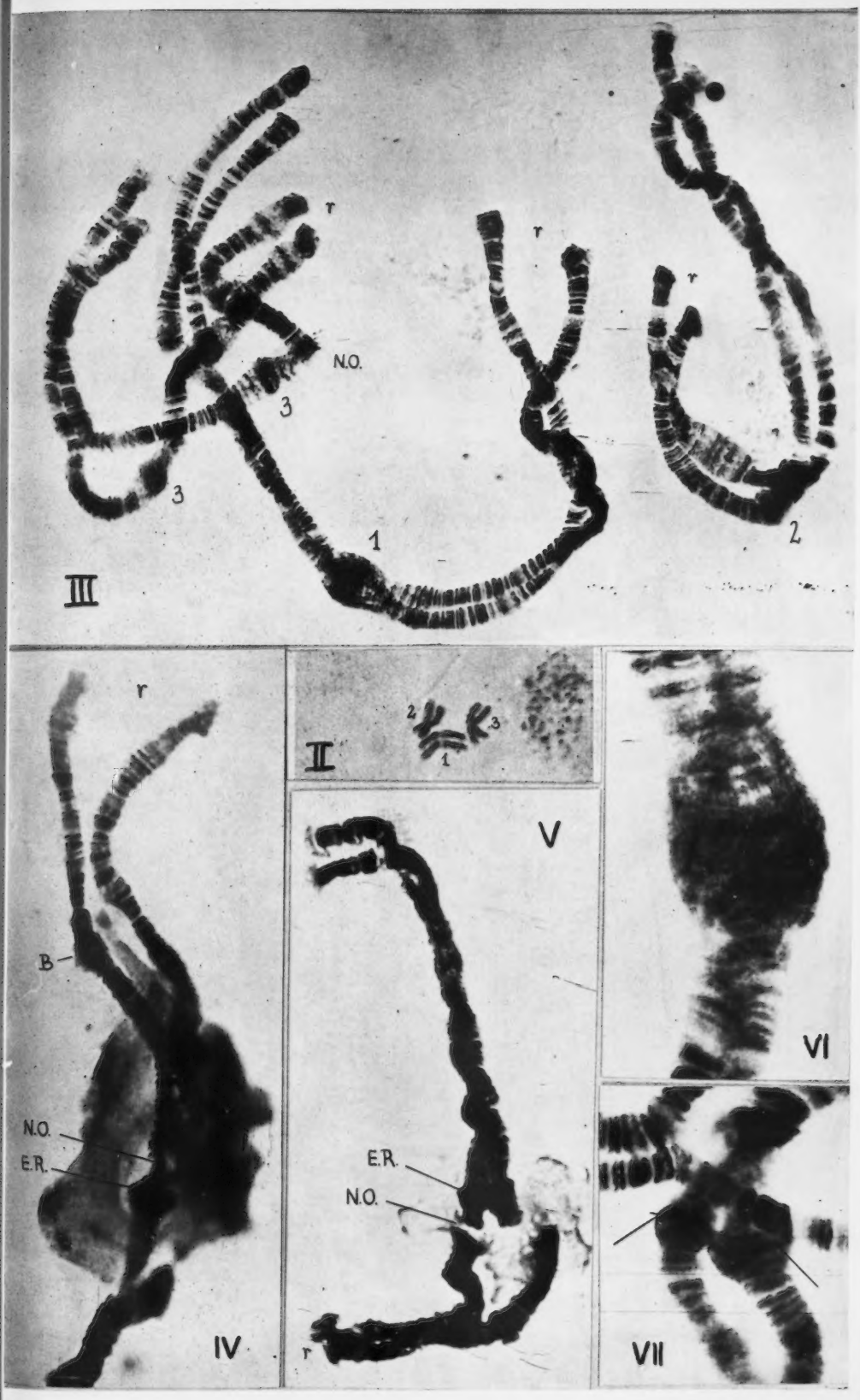


FIG. I. Salivary gland chromosome idiogram of *S. vittatum* based on the mean % TCL of Table I and indicating the expanded regions, nucleolar organizer (*N*), Ring of Balbiani (*B*), and incomplete pairing.

Figs. II–VII.

FIG. II. Neuroblast metaphase and interphase. Numbers identify chromosomes and centromeres. FIG. III. Complete salivary gland complement of animal 375. Chromosomes identified by numbers at the expanded regions. Right ends (*r*) and nucleolar organizer (*NO*) indicated. FIG. IV. Right arm of Chromosome 3 counterstained with fast green to show the expanded region (*ER*), nucleolar organizer (*NO*) and nucleolus, and Ring of Balbiani (*B*). FIG. V. Chromosome 3 showing nucleolar gap. FIG. VI. Expanded region of Chromosome 1 (No. 375) with indications of longitudinal unbranched fibrillae. FIG. VII. Expanded region of Chromosome 3 (No. 459) with exceptional banding (arrows).


r
N.O.
3
3
1
r
r
2
III
II
2
3
1
V
E.R.
N.O.
r
VI
VII
r
B
N.O.
E.R.
IV

A full discussion of theories of salivary gland chromosome structure is not in the scope of this paper. The authors are in substantial agreement with the view, propounded especially by Bauer (1, 2, 4), that salivary gland chromosomes are bundles of chromonemata the juxtaposition of chromomere-like bodies resulting in chromatic disks or bands separated by achromatic interband regions. As expected the salivary gland nuclei of *S. vittatum* show three giant chromosomes, each with two loosely wound constituents (Fig. III).

Each salivary gland chromosome is subdivided into two arms by a characteristic "expanded region" (numbers in Fig. III). Since there is only one such region per chromosome and since the resulting arm ratios conform with those of ganglion chromosomes of like relative size, expanded regions of salivary gland chromosomes and centromere regions of neuroblast chromosomes are homologized. A chromocentric fusion of centromere regions is lacking in the salivary gland nuclei. In this respect *Simulium* resembles *Bibio* (18), *Culex* (20), sciarids (24), cecidomyids (37, 38), and chironomids (2) and differs from *Anopheles* (13) and most species of *Drosophila* except *robusta* (8).

Chromosome 3 differs from No. 2 not only in the arm ratio but also in the possession of two nucleolar structures. The single large nucleolus attaches near the expanded region and a second granular structure comparable to the Ring of Balbiani of *Bibio* and *Chironomus* is located near the center of the nucleolus bearing arm.

The chromosomes of one complete nucleus were measured from each of five *vittatum* larvae representing three different localities. The individual and

TABLE I

MEASUREMENTS* OF ONE COMPLETE SALIVARY GLAND COMPLEMENT FROM EACH OF FIVE LARVAE REPRESENTING THREE DIFFERENT LOCALITIES

Animal	Chromosome segment (% TCL)									Complement in μ
	1R	1L	2R	2L	3R-Balb.	Balb.-N.	N. Gap	N. Exp. R	3L	
375 Don River	21.8	21.5	10.2	19.1	6.0	4.0	(1.0)	1.1	16.3	501
209 Churchill	18.9	18.6	10.9	19.0	7.3	4.9	(0.6)	1.5	18.8	475
459 Collingwood	20.4	17.5	11.0	18.6	7.5	4.6	(0.5)	1.5	18.9	825
386 Don River	20.9	17.3	11.1	20.8	5.9	5.0	(0.8)	1.5	17.5	752
361 Don River	24.6	16.3	10.9	21.1	6.2	4.2	(3.4)	1.0	15.6	702
Mean	21.3	18.2	10.8	19.7	6.6	4.5	(1.3)	1.3	17.4	

** Dimensions of chromosome arms and major subdivisions expressed in % total complement length (excluding nucleolar gap).*

average measurements are shown for major chromosome segments in per cent total complement length (% TCL) in Table I. The nucleolar gap, which is exceedingly susceptible to differential stretching, is not included in total complement length. The average values form the basis of the salivary gland chromosome idiogram of Fig. I which shows arm ratios, position of diagnostic landmarks, and a diagrammatic representation of incomplete pairing. Additional nuclei were measured but yielded no significant refinement of average % TCL values.

In accordance with *Drosophila* usage the two arms of each chromosome were labelled "left" and "right". We choose to call the shorter arm "right" (Fig. III: *r*). In describing chromosome regions the ternary system introduced by the Bridges (6, 7) was followed. The entire complement was divided into 50 sections (1, 2, 3, . . . 50) beginning with the right end of each chromosome. Each section was divided into a number of divisions (*A*, *B*, *C*, etc.) in which individual bands were numbered consecutively (1, 2, 3, etc.). Thus the first band would be 1*A*1, the second 1*A*2, etc. Limits of sections and divisions only are indicated in the photographic maps (Figs. VIII–XXXII), and for the purposes of the present paper the binary mode of reference was generally adequate. Maps of Chromosome 1 are found on Plate II, those of No. 2 on Plate III, and those of No. 3 on Plate IV.

The expanded regions excepted, the diameter of the salivary gland chromosomes is approximately uniform, but certain constrictions or "necks" can almost always be recognized. These include the regions 1*F*, 3*A*, 10*B* on Chromosome 1; 24*C*, 29*A* on Chromosome 2; and 37*F* and 45*A* on Chromosome 3.

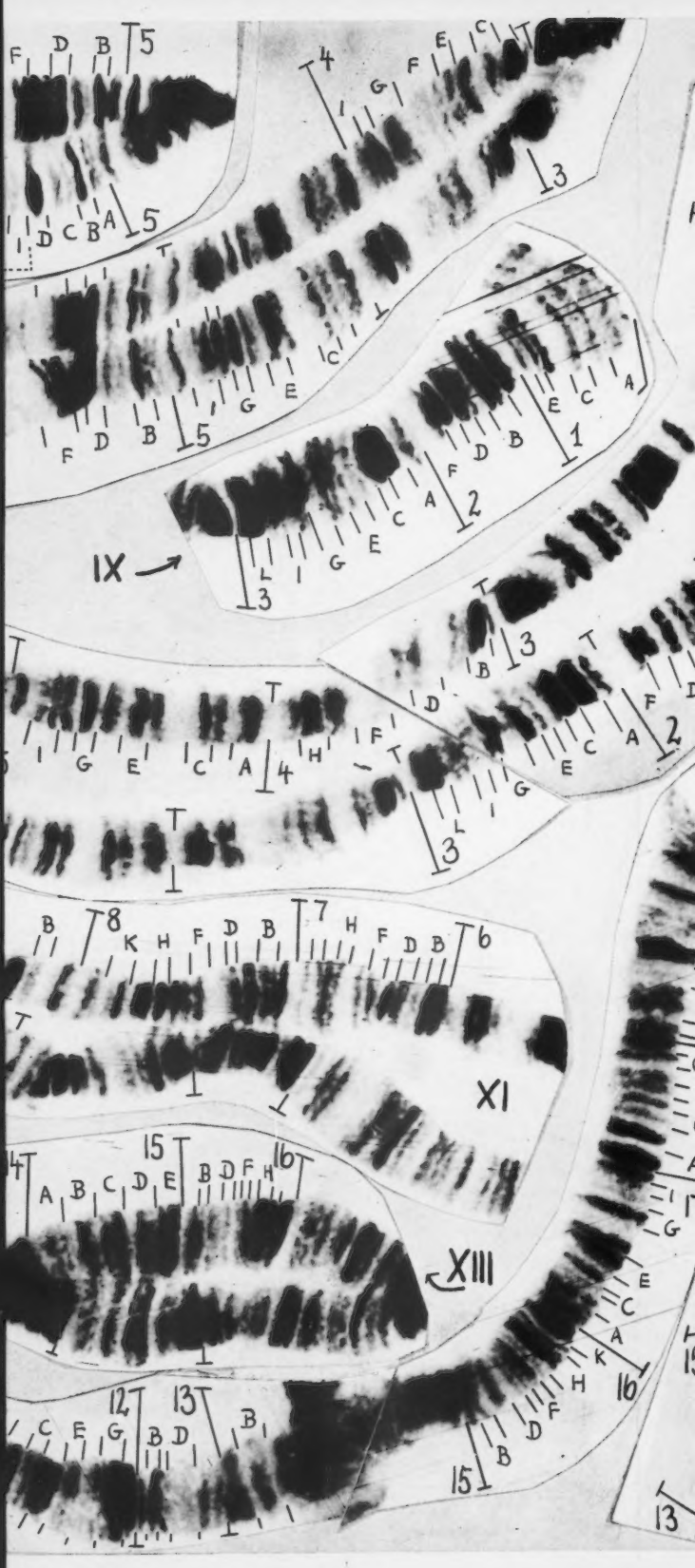
In *Simulium* as in all other Nematocera except *Anopheles* (14) and mycetophilids (21) consistent chromosomal differences between the sexes could not be found either in neuroblast or in salivary gland nuclei.

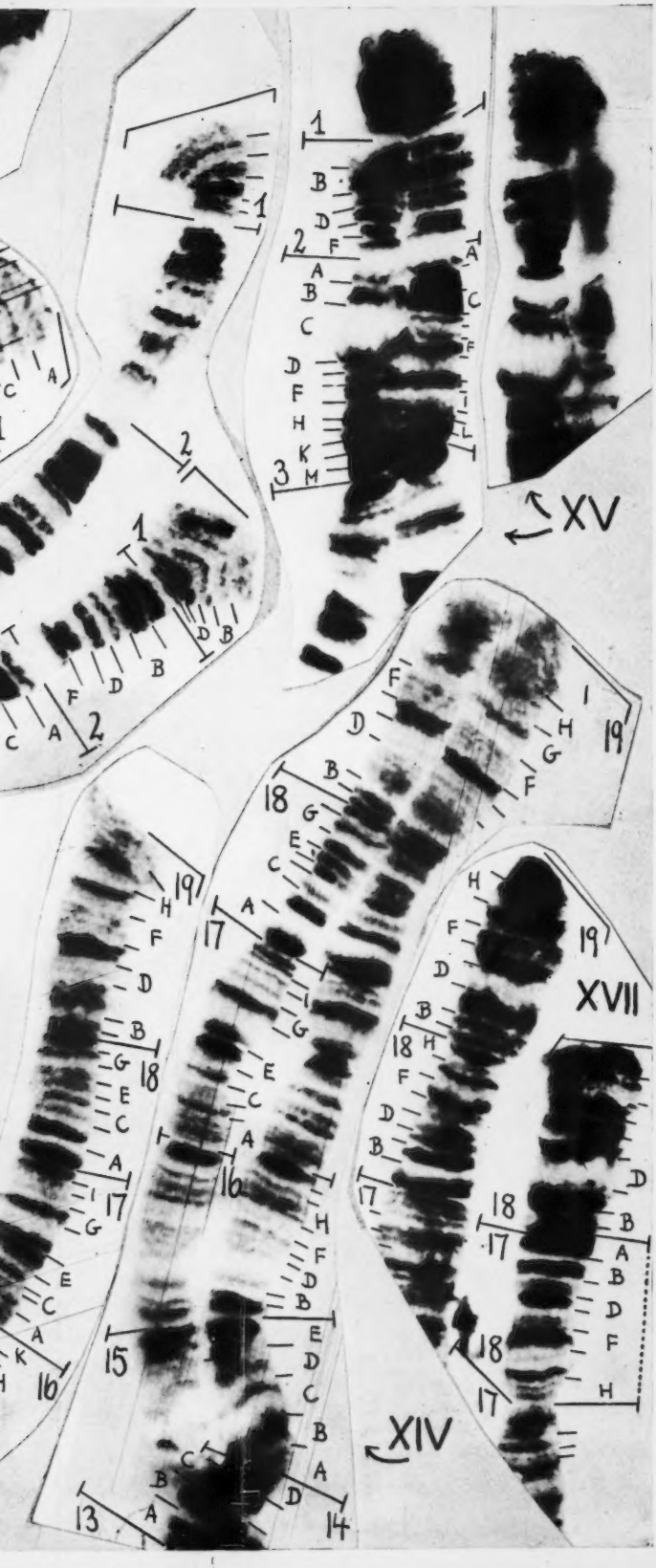
Use of chromosome numbers and arm ratios alone as species diagnostics does not do justice to the peculiar advantages of this material. In a broader evolutionary context the following observations are of value. As far as our experience goes the mitotic neuroblast complements of black flies are surprisingly constant. Not only is the haploid number $n = 3$ nearly universal but also the relative lengths and arm ratios of chromosomes are preserved through a long series of species. There are two notable exceptions to this uniformity. *Eusimulium euryadminiculum*, like *Cryptochironomus* spec. 2 (3), has only two chromosomes in the haploid set. This complement possibly evolved from the

Chromosome 1 (Sections 1–19). Figs. VIII–XVII.

FIG. VIII. Animal 375. Don River, Toronto, 19.2.52. Both constituents. Sections 1–6, 9–12. FIG. IX. Not numbered. Humber River, near Toronto, 10.3.52. One constituent. Sections 1–3. FIG. X. No. 375. Both constituents. Sections 4–9. FIG. XI. Animal 371. Don River, Toronto, 10.2.52. Both constituents. Sections 7–12. FIG. XII. No. 371. One constituent. Sections 10–13, 16–19. FIG. XIII. No. 375. Both constituents. Sections 15 and 16. FIG. XIV. No. 371. Both constituents. Sections 14–19. FIG. XV. Animal 361. Don River, Toronto 3.2.52. Two different focal levels. Both constituents. Heterozygous for "pumpkin-like" modification of standard bands 3*C*. FIG. XVI. No. 361. Lower constituent with inversion from 6*E*–6*I* (broken line), upper constituent standard. FIG. XVII. No. 375. Right constituent with inversion of segment 18, left constituent standard.

XVI
X
IX
VIII
XII


IX
XI
XIII


XV
XIV
XVII

standard type by pericentric inversions of two chromosomes, followed by an unequal interchange and loss of a small fragment. In *Cnephia mutata*, in this latitude, upwards of 85% of the individuals are triploid. This and the abnormal sex ratio (11) indicate the significance of parthenogenesis in the life cycle of this species. Because of the isolated taxonomic position of the genus, great interest attaches to the complement of *Gymnopais* which so far has not been studied.

II. Characteristics of the Expanded Regions

As mentioned above each of the salivary gland chromosomes of *S. vittatum* carries a strikingly prominent expanded region. In all three chromosomes this typically appears as in Figs. VIII, XVIII, XXX, as a region with one and a half to three times the diameter of the remaining chromosome and with a loose large-chromomeric or tufted appearance. Careful focusing suggests that these large chromomeres occur in all parts of the optical section and are not merely peripherally disposed. Particularly in slightly stretched specimens (Fig. VI) it can further be seen that the expanded regions are traversed diagonally by fibrillae which carry chromomere-like bodies without branching or lateral outgrowths. We therefore interpret the expanded regions as being essentially like the rest of the chromosome except that the structure is locally modified by excessive size of chromomeres, lack of attraction between them, or overdevelopment of "matrix", resulting in a local blowing up of the chromosome and disaggregation of bands. Additional evidence for the correctness of this view comes from the observation of occasional atypical expanded regions in which the normally disaggregated chromomeres appear organized into one very heavy central band and one or two lighter ones on either side (Fig. VII). The typical picture results then from the more complete disorganization of approximately five bands. Occasional individuals were found which were heterozygous for expression (or structure ?) of this region, one constituent showing typical bands, the other—of wider diameter—showing disordered chromomeres.

A number of other *Simulium* species including *pictipes* and *decorum* have "compact" expanded regions with loose chromomeres very much like those of *vittatum*. The compact expanded regions of certain additional *Simulium* (*tuberosum*, *venustum*) and *Eusimulium* (*aureum*, *latipes*, *croxtoni*) and of Geitler's unidentified species are distinguished by more or less clearly expressed heavy central bands. Radically opposed to these compact types are the long "cottony" expanded regions first described by Painter and Griffen (23, Fig. 7) for *S. virgatum** and observed by us in some *Prosimulium* (*hirtipes*), *Cnephia*

* *Considerable importance attaches to the correct identification of P. and G.'s material. The authors merely state that most of their work was done with S. virgatum; it is probable that this includes their extensive study of the "spread out" region. It is likely that the species identified by them as Simulium (Dyarella) virgatum Coquillet was actually a closely related form later described by Stone (31) as S. (Dyarella) solarii. Virgatum and solarii are the sole known North American members of the tropical subgenus Dyarella, closely related to the subgenus Simulium. Accepting this identification, the cottony type of expanded region occurs in all four investigated genera: Prosimulium, Cnephia, Eusimulium, Simulium (Dyarella), where generic rank is allowed for Eusimulium.*

(*mutata*, *dacotense*), and *Eusimulium* (*innocens*, *furculatum*). The large amorphous deeply staining expanded regions of *E. euryadminiculum* are somewhat intermediate between the cottony and the compact type.

Our preliminary survey indicates that the occurrence of expanded regions on at least one chromosome is universal in black flies and that the specific characteristics of these regions vary widely from one species to another. Thus they will be eminently useful in describing segregates.

Expanded regions resembling those of *S. vittatum* occur outside the simuliids, those of the chironomid *Glyptotendipes polytomus* being strikingly similar (2). Other chironomids such as *Chironomus Thummi* (1) and *Cryptochironomus defectus* (2), originally described by Bauer as having "compound heterochromomeric bands", have expanded regions quite comparable to those of *S. tuberosum*, while yet others like *C. tentans* (5) and *Tendipes decorum* (12) allow no obvious morphological identification of centromere regions. Salivary gland chromosomes of several sciarids (24) and cecidomyids (38) show deeply staining compact portions which it is tempting to homologize with centromere regions, however in certain species (*Taxomyia taxi*, *Dasyneura affinis*, etc.) more than one such structure is found per chromosome.

Obviously, when attempting to interpret relationships between different simuliid species from characteristics of the expanded region, caution is needed in two respects. Firstly, since salivary gland centromeres of certain species are not morphologically distinct, granting that centromeres within the Diptera are fundamentally alike, it seems probable that expanded regions of the *vittatum* type are not centromeres per se but centromere associated "heterochromatin". Since heterochromatin of a similar type might conceivably occur away from the centromere, and evidently does at least in cecidomyids, expanded regions of different forms are not necessarily homologous to centromeres or to each other. However where there is only one such region per salivary gland chromosome and where it conforms in position to the constriction of ganglion chromosomes presumptive evidence for identity is good. Further corroboration derives from the failure of inversions—common in most black fly species—to span the expanded region in even a single instance.

Secondly, since centromeric heterochromatin may appear alike in members of different families (*Glyptotendipes* and *Simulium*) and unlike in species of a single genus, its extent and expression are evidently phylogenetically fairly plastic and care needs to be taken not to stipulate evolutionary affinities on the basis of similarities in these regions alone. Neither of these two qualifications invalidates the usefulness of these regions in the recognition of species in black fly larvae and limited conclusions about their interrelations may be possible.

III. The Main Nucleolus

When intact salivary glands of *S. vittatum* are mounted in "Nujol" and observed in the living state, a single large smooth and commonly bilobed nucleolus is visible in every nucleus. The same is true for all other simuliids examined by us so far, as well as for the species studied by Geitler (17). In

Levitsky–Feulgen – fast green preparations the nucleolus is preserved very well (Figs. IV, V) and in material from all localities occupies the same site near the expanded region of the third chromosome. The attachment region varies in the extent of disorganization. In many instances the constituents flare apart into unequal trunks which subdivide again and again until they disappear—with variable clumping—in the nucleolar mass. In other cases (Figs. XXVIII, XXIX, XXX) the banding is almost completely undisturbed when it is apparent that the interband region 41 *F-G* acts as the center of nucleolar organization. In yet other cases the attachment point is characterized by a nonstaining gap (Fig. V).

Within these limits of variation, expression of nucleolar regions is alike in all simuliids studied but the position of the nucleolar organizer within and between chromosomes varies from species to species. In the majority of forms the nucleolus is found in the longest chromosome, generally near the center but terminally in *S. pictipes*. In the remaining species (generally *Simulium* exclusive of *pictipes*) the nucleolus occurs near the expanded region of one of the shorter chromosomes and either in the long or the short arm.

Rings of small "Nebennucleolen" found by Bauer in *Chironomus Thummi* (1) and by us in various chironomids probably also occur in simuliids in association with characteristic alveolar sites. No attempt was made to map these regions in *S. vittatum*.

Because of the intraspecifically constant and interspecifically variable attachment of the main nucleolus, study of nucleolar relations in simuliids promises to be diagnostically useful. The remote possibility of homozygous perinucleolar inversions on a subspecific level must be borne in mind. Chironomid larvae of the difficult genera *Tendipes* and *Camptochironomus* of the "plumosus type" may be distinguished by the number of nucleoli alone, the former having one and the latter two (3). Various mononucleolar forms (*C. dorsalis*, *C. bathophilus*, *C. plumosus*) can be differentiated by the location of the nucleolar organizer (2).

In the chironomids Bauer (2) feels that, because of the wide differences in nucleolar numbers in different species and their possibly independent evolution from the numerous equipotential nucleoli of forms like *Stictochironomus histrio*, nucleolar organizers of different species cannot safely be homologized. This may also be true for the cecidomyids (38), which include a number of binucleolar forms and in any case exhibit enormous cytological diversity. In a strictly mononucleolar group such as the simuliids one need be less apprehensive in this regard and quite possibly within the group nucleolar organizers have not arisen de novo but are interderived by inversions and interchanges alone. This theoretical possibility finds some practical support in the discovery of perinucleolar inversions in *Chironomus dorsalis* by Philip (25) and in *Simulium decorum* by Miss Zimring in our laboratory. It may further be possible to substantiate this hypothesis by interspecific band comparisons of the nucleolar attachment regions. In the meantime the main nucleolus in the salivary gland complement remains a ready diagnostic of taxonomic segregates.

IV. The Ring of Balbiani

In the salivary gland nuclei of *S. vittatum* and most, if not all, other species of black flies so far examined, an additional type of nucleolar structure is found. This is small and inconspicuous in living nuclei; in stained preparations of *S. vittatum* the attachment point is blown up and large-chromomeric, somewhat like the expanded region, but in other species apparently equivalent organizers may be expressed like the main nucleolar attachment or like the special disbanded regions described by Bauer and Beermann (4, 5) for *Chironomus tentans*. Nucleolar substance—as judged by fast green response—is laid down in small amounts on these modified chromatic portions and in *S. vittatum* appears to be condensed on individual chromomeres giving the structure as a whole a mulberry-like appearance (*B* of Fig. IV). Since these regions morphologically resemble the structures termed "Rings of Balbiani" in *Bibio* (18) and in various chironomids (1, 2, 4, 26), this term will be applied to them.

The site of the Ring of Balbiani is species specific, thus affording another landmark in the salivary idiogram (Fig. I). In *S. vittatum* it is located in the same arm as the main nucleolus, it involves the region 39*A–B* (Figs. XXVI-XXVIII) and is apparently the center of a symmetrical reverse duplication. This is inferred from the not infrequent lateral pairing of presumably homologous bands on either side of the Ring of Balbiani resulting in a cuplike effect (Fig. XXVI, upper constituent, and XXVII).

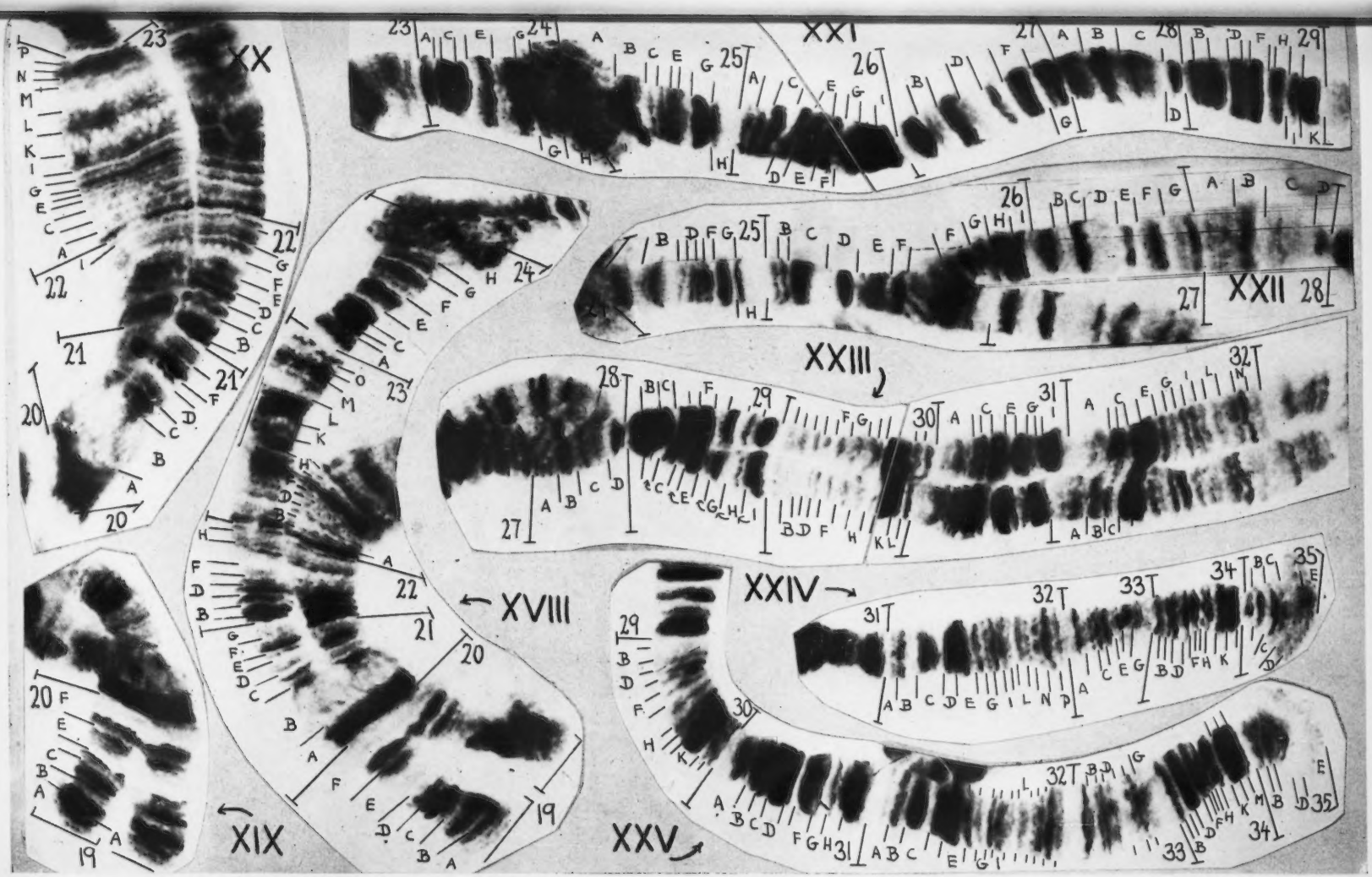
Although this region in *S. vittatum*, *P. hirtipes*, *S. pictipes*, and others is unique, readily identifiable, and thus a good species character, the situation in some *Eusimulium* is more obscure. Thus in *E. euryadminiculum* there is no single obvious counterpart but a number of regions grading down from typical Balbiani-like stuctures to barely modified heterochromatin (see below). Further work with living nuclei and nucleolar stains is required before a more rigorous definition and classification of these regions can be attempted and before convincing notions can be developed as to their interspecific homologies.

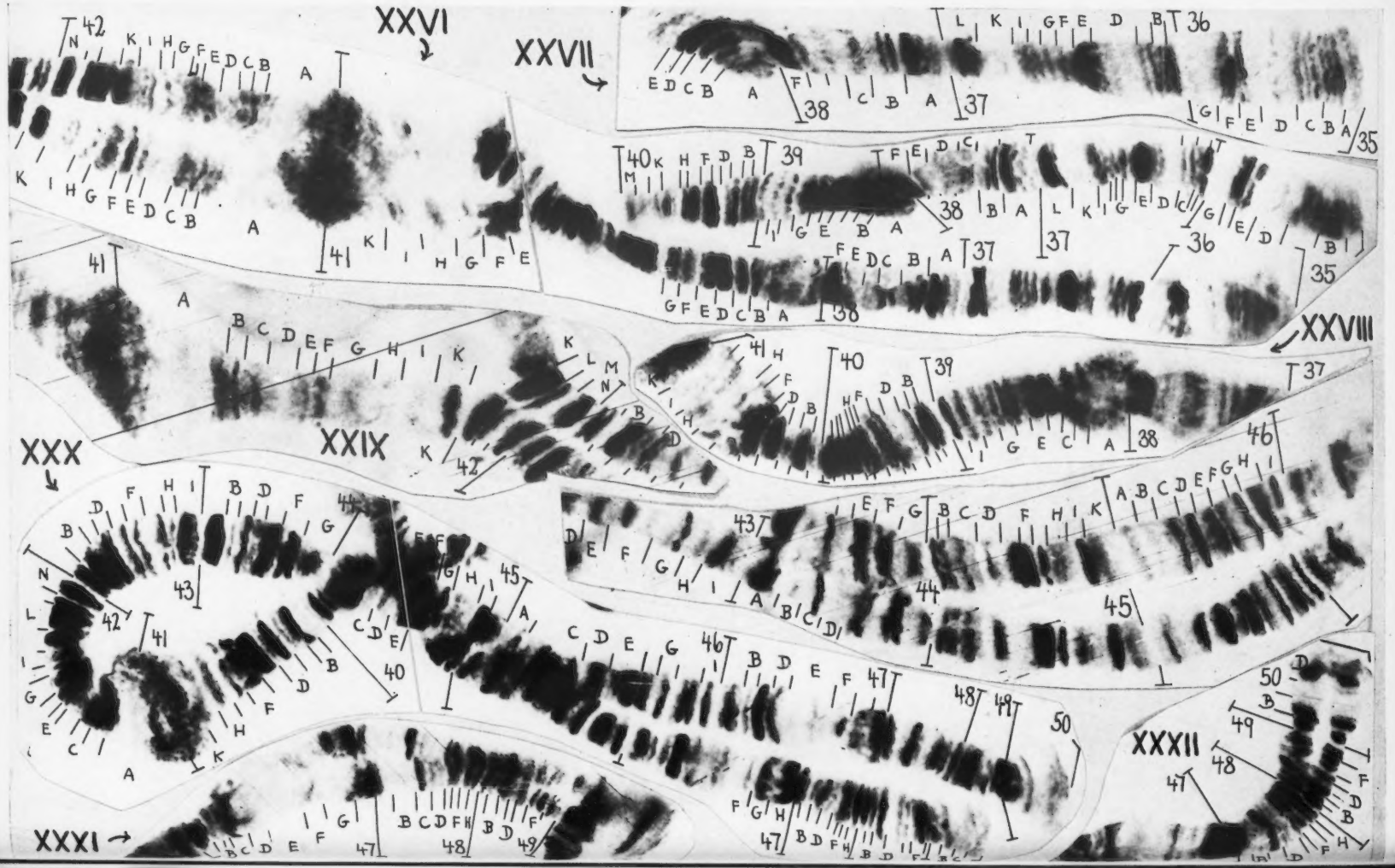
V. The Degree of Pairing

In *S. vittatum* from all localities, pairing of homologous constituents is very loose, unlike that described for most *Chironomus* (2), *Bibio* (18), *Anopheles* (13), *Culex* (20), cecidomyids (38), and sciarids (24), and like that described for *Trichotanypus pectinatus* and other Tanypinae (2). An extensive survey of the pairing relations in *S. vittatum* was not attempted with squashed material but certain generalizations are quite evidently valid.

Chromosome 2 (Sections 20–35). Figs. XVIII–XXV.

FIG. XVIII. No. 375. Both constituents (±). Sections 20–24. FIG. XIX. No. 375. Both constituents. Section 20. FIG. XX. No. 375. Both constituents. Sections 21–23. FIG. XXI. No. 375. One constituent. Sections 24–29. FIG. XXII. No. 371. Both constituents (±). Sections 25–28. FIG. XXIII. No. 375. Both constituents. Sections 28–32. FIG. XXIV. No. 375. One constituent. Sections 32–35. FIG. XXV. No 375. One constituent. Sections 30–35.


XX
XXI
XXII
XXIII
XXIV
XXV
XVIII
XIX


XXVI
XXVII
XXVIII
XXIX
XXX
XXXI
XXXII

(1) The degree of pairing is approximately alike in lowest and highest degree polytene chromosomes; thus an equilibrium condition is established early and is not affected by further chromonema reduplications.

(2) Lack of close pairing is not generally due to demonstrable structural heterozygosity. On the other hand, of course, the general looseness of pairing interferes with the characteristic expression of the occasional region with respect to which the larva was a structural hybrid. Thus inversions, unless they are extremely long, do not lead to typical reversed loops when heterozygous (Figs. XVI, XVII).

(3) Failure of close synapsis may occur in any part of the complement but a statistical study would probably reveal regional differences in liability to pairing. Thus in Chromosome 2 the regions 21*A*, 29*A*, and 32*D* are almost always intimately paired. Preferential pairing appears to be based on very heavy bands or groups of heavy bands but not all heavy bands function in this way. Somewhat contrary to expectation the expanded regions are frequently not synapsed (Figs. VIII, XVIII, XXX). Furthermore, there is no evidence for nonhomologous attraction of these centromere regions. More or less random terminal or semilateral attachments of chromosome ends to one another are not uncommon.

Salivary gland chromosome pairing, as weak as that of *S. vittatum*, was also observed by Geitler in his unidentified species and, to a lesser extent, by Painter and Griffen in *S. virgatum*. On the other hand we have encountered forms in which pairing is so close that the double nature of salivary gland chromosomes is obscured. Intermediate types exist. Each species appears to have a characteristic pairing norm, widely different from that of at least certain other species, and thus a useful adjunct in species identification. The distribution of completely paired forms as observed so far is quite erratic and cuts clear across present taxonomic boundaries including as it does some *Eusimulium* (*furculatum*, *euryadminiculum*, *innocens* but not *latipes*, *croxtoni*, *aureum*), some *Cnephia* (*dacotense* but not *mutata*), at least one *Simulium* (*pictipes*), and *Prosimulium* species to varying degrees. Study of yet further forms should enable one to decide whether this seemingly erratic distribution signifies convergent development of pairing relations in several independent evolutionary lines or whether it is caused by spurious association of unrelated forms in taxonomic artefacts. In the only comparable case on record incomplete pairing within the chironomids is confined to the Tanypinae according to Bauer (2).

Chromosome 3 (Sections 36–50). Figs. XXVI–XXXII.

FIG. XXVI. No. 375. Both constituents. Sections 36–42. FIG. XXVII. No. 375. One constituent. Sections 36–38+. FIG. XXVIII. No. 375. One constituent. Sections 39–41. FIG. XXIX. No. 371. Both constituents (±). Sections 41-46. FIG. XXX. No. 375. Both constituents in part. Sections 40–44, 46–50. FIG. XXXI. No. 459. Collingwood. 15.4.52. Both constituents. Sections 47–49. FIG. XXXII. No. 459. Both constituents. Sections 48–50.

VI. The Banding Pattern

In our experience in Feulgen stained Carnoy material the typical band is like those of regions 2*CD* (Figs. VIII, IX, XV) and 34*EFG* (Fig. XXV), a unitary chromatic structure and not vesicular, bubbly, capsular, or vacuolated as described by Painter and Griffen (23) for both living and fixed chromosomes. We are inclined to consider the universal bubbly nature of bands in their work as a significant artefact comparable to acetic acid vacuolation of root-tip chromosomes. We have obtained similar images after prolonged submortem exposure of glands to hypotonic media.

The typical band or disc, as it also appears in chironomids, we will call euchromatic; it may be heavy or light, sharp or fuzzy, solid or broken up to varying degrees. The distribution in the complement of heavy and light euchromatic bands is not random, but apparently reflects some over-all organization. Large numbers of fine bands are found around the expanded regions of all three chromosomes (10*L*–11*B*, 24*C*–26*B*, 41*F*–42*K*) and at certain intercalary sites such as 22*F*–23*G* and 30*A*–30*I* of No. 2 chromosome. Bona fide doublets of the *Drosophila* type do occur and not infrequently heavy "double doublets" are found such as 6*AB* of Chromosome 1 (Fig. X).

There occur in *S. vittatum* a number of chromosome regions which are more or less clearly distinct from euchromatin and these—in deference to general salivary gland chromosome usage—will be referred to as heterochromatic. In the absence of any information on further cytological or genetic attributes it is altogether probable that this heterochromatin is a heterogeneous assemblage and in fact all conceivable intermediates are found between quite unorthodox chromosome segments and typical euchromatin.

Common denominators of heterochromatic regions are greater variability in expression; wider diameter; loose, fuzzy, or alveolar banding, and potentially an exploded or blown-up condition realized most conspicuously in deeply stained prepupation chromosomes.

Most heterochromatic regions involve several similar band groups resulting in a block with a measure of unity. Typical examples are 8*K*–9*A* (Figs. X, VIII, XI), and 43*EFG* (Fig. XXX). Regions such as 5*ABC* (Fig. X), 8*DEF* (Figs. X, XI), 11*FGM* (Fig. VIII), 12*EFG**, 13*DE*, 15*BCD*, 42*GH*, and 45*ABC* (Fig. XXIX) are of the same general type but less pronouncedly heterochromatic. Certain additional heterochromatic regions, which like 4*C*–4*F* (Figs. VIII, X), 15*AB*, 28*BCD* (Figs. XXI–XXIII), and 44*D*–44*G* (Fig. XXIX) are spindle-shaped in undistorted chromosomes, seem particularly liable to differential stretching.

On the other hand heterochromatic characteristics may reside in individual bands or seeming interband regions. Such single alveolar bands may vary from the extremely heavy sawtooth of 47*GH* (Fig. XXX) through the conspicuous alveoli of 23*K* (Fig. XX) and minutely pearled bands like 22*G*

* *Figures are only cited where the illustrations selected for the plates show heterochromatic characteristics with reasonable clarity.*

(Fig. XX) till they merge imperceptibly into euchromatic bands. They are morphologically suggestive of regions which in chironomids form rings of "Nebennucleolen".

Finally the predisposition to "explode" appears to form a modal characteristic of a further class of heterochromatic regions. A conspicuous representative is 47*DE* (Figs. XXX, XXXI) which may involve neighboring bands to a variable extent. Lesser regions of this type are 2*F*-3*A* (Figs. IX, X), 8*DEF* (Fig. XI), 19*D* (Fig. XVII), 20*F* (Figs. XVIII–XX), 21*B* (Figs. XVIII, XX), 32*O*-33*A* (Figs. XXIII, XXV), and 36*D* (Figs. XXVII, XXVIII) with a conspicuous broken band through the center.

The photographic maps of Figs. VIII-XXXII show practically all chromosome regions of *S. vittatum* at least twice. Duplicate maps are drawn in part from different nuclei of the same larva, in part from different larvae of one collection, and in part from larvae from different localities (see figure explanations for details). Limits of sections and divisions only are indicated in the maps. Throughout, salivary gland chromosomes showed much better agreement in the general properties of staining, stretching, separation, and band definition when the comparison was made of larvae from one collection rather than of larvae collected at various localities or times. On the other hand once the maps were drawn up, limits of division would fairly readily be recognized regardless of the geographic origin and occasional quite foreign appearance of chromosomes. The regions 47–50 of No. 371 from Toronto and of No. 459 from Collingwood (Figs. XXX–XXXII) illustrate the extremes of diversity encountered. In this limited study consistent geographical differences were not found although the heavy end band 50*D* of No. 3 from Collingwood (Figs. XXXI, XXXII) was not observed in Toronto material.

Only one large inversion was encountered (in the heterozygous condition) involving roughly the regions 43–47, but smaller inversions and undefined structural changes involving only a few bands were very common. It is not proposed to describe in detail all that were worked out. The following examples from Chromosome 1 are representative: Animal 361 carried a heterozygous inversion in the right arm with the breakage points 5*D*2 and 6*I*2 (Fig. XVI). Animal 375 carried a heterozygous inversion in the opposite arm with breakage points coinciding almost exactly with the limits of Section 18 (Fig. XVII). Animal 361 was also heterozygous for a very characteristic pumpkin-like modification of standard bands 3*C* (Fig. XV) clearly expressed in all analyzable nuclei. Pairing relations were not disturbed in any of these cases. All larvae that were carefully examined proved to be structurally hybrid to some extent. In *S. vittatum*, in contrast to *E. aureum*, no structural changes were found involving chromosome ends.

Comparison of the ultimate resolvable banding pattern will provide the final criteria of species identity. It appears that the "species" examined so far are so clearly segregated cytologically that preparation of complete maps will not in general be necessary. Mapping of chromosome ends and of

nucleolar and Balbiani settings along with gross characterization of chromosome morphology may suffice for species description. This is not to be construed as a clairvoyant assertion that further work, especially on a wider geographic scale, will fail to reveal reproductively isolated entities within the aggregate defined by these cytological criteria and of either specific or subspecific rank.

The ultimate goal of phylogenetic work—the comparison of band sequence in different species—may be difficult to accomplish in black flies. Excepting *Cnephia dacotense* black flies are not stenogamous, mating flights are required, diapause intervenes, and larvae are physiologically exacting in their requirements. Thus the prospects of fertile interspecific matings and the determination of segmental homologies from an analysis of chromosome pairing in hybrids are slight. Nonetheless such crosses should certainly be attempted with *C. dacotense* as one parent. The other possible approach—search for interspecific homologies by comparison (inspection, drawing, photography) of the banding pattern of wild species—perhaps holds some hope for success at least in closely related forms and where the comparison is of chromosome ends or other prominently characteristic regions.

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THE NATURE OF CYCLES IN POPULATIONS OF CANADIAN MAMMALS[1]

By L. Butler

Abstract

The number of pelts of the various fur animals collected each year since 1915, are examined critically to determine whether the peak or high collections are cyclic, or whether they could arise by chance in a series of random numbers as suggested by Cole. Cole's criterion of a peak is rejected as impractical and misleading. By splitting the country into 53 sections, it is shown that peaks do not occur at random as would be expected if these are chance occurrences. Nor do the peaks occur in the same year all over the country, but rather they are spread over three to four 'good' years which are followed by five or six 'bad' years, when no peaks occur in any section. Peak collections for the whole of Canada are the resultant of simultaneous high collections in most parts of the country. Rarely are all the sections of the country in phase; whenever a high-producing section gets out of phase it may produce a secondary peak, or even a split peak in the total for the whole country. It is shown that there is a relationship between the number of sections experiencing a peak, and the peak for the whole country. In general, the peak collection of most furs is reached first in the central provinces, then in the western, and finally in the eastern provinces. Data from annual questionnaires confirm this sequence. They show that the increase phase for snowshoe rabbit, mink, and fox begins as an island of increase in the prairie provinces surrounded by an area of decrease or no change. The reports of increase on subsequent years can be plotted by isophasal lines which spread out like waves from the original area of increase. The easiest interpretatation for these waves is migration from the areas where increase began into the more sparsely populated surrounding sections. These migrations would have a synchronizing effect on the population changes over the whole area. They would also give hybrid vigor to the local population by mixing gene pools which have been isolated for several generations. It is suggested that the causal agent of these cycles is the accumulation of favorable factors in good years, acting over most of the country on populations of different initial densities. Migration from the more favored areas will cause an upswing of the cycle over most of the range. Decrease, being largely density-dependent, will follow across the country in the same order.

Introduction

Populations of animals and plants fluctuate in size from year to year, and over the past 30 years the theory has developed that such changes exhibit enough regularity to be classed as periodic or cyclic. In 1924, Elton (9) postulated that fur returns for Canada (number of pelts collected annually) varied greatly from year to year, and that such changes were of a periodic nature. Two main types of periodicity were found in Canada; the 10-year cycle of the bush country, and the four-year cycle of the arctic. The presence of these cycles was generally accepted, and a great deal of effort has been expended trying to find cycles in all kinds of data. Research workers have reported population cycles in many small mammals, gallinaceous birds, insects, and fish; while still others have found similar cycles in the rate of tree growth, frequency of disease epidemics, and even in such human events as revolutions, birth of geniuses, and economic depressions.

If one is justified in concluding that populations do exhibit regular, predictable fluctuations, then it should be possible to forecast future yields of fish, game, and fur. Since this is of practical importance, and would be a step

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towards management of our resources, there has been a tendency to accept such periodicities without a critical study of what constitutes a cycle. Basic research has also been undertaken on the causes of these cycles without establishing that cycles really exist, and are not something which has been read into a series of random fluctuations. Recently, Cole (6) has been critical of the general acceptance of cycles and has suggested that they may be found in random or chance series. So it behooves us to go back and critically examine our source material in the light of the suggestions of Cole and other critics.

The literature on population cycles in animals is well summarized by MacLulich (14), Elton (9, 10), Dymond (8), Siivonen (17), Clarke (5), and Rowan (16). These authors tend to divide the animals which participate in cycles into two classes: prey animals such as mice, rabbits, lemming, and grouse; and predators such as lynx, fox, owls, and hawks. The main assumption in the literature is that the prey cycle is the basic one and that the predator cycle merely follows the prey one. Cole makes similar assumptions without critical examination of the evidence. We shall see later that the predator/prey relationships are not as constant as is generally accepted. Furthermore, the quantitative data available for prey animals are extremely limited. This is probably the reason why so many of these relationships have been accepted.

To find out whether the population changes take place with enough regularity to be classed as cycles, we have available two kinds of data: the annual collection figures for the various kinds of pelts, and the annual questionnaires of the relative abundance of certain animals. Each kind of data uses a different criterion in assessing the state of the animal population; the data from pelt returns assume that the number of pelts taken is proportional to the population present, while the questionnaire data assumes that the number observed this year, compared with the number observed in the previous year, shows the relative change in the population. The material available of each type, together with a brief appraisal of its suitability for this investigation, is given in the following paragraphs.

The pelt collection data come from two sources, the Hudson's Bay Company and the Dominion Bureau of Statistics (7). The H. B. C. material consists of pelt returns by species for the whole of Canada for the years 1821–1952. For the period 1821–1915 it is impossible to subdivide the total collection for Canada into returns for definite sections of the country. After 1915, the pelt collection can be broken down to the individual collecting points or posts of which there are some 200 scattered across the country. Each of these posts draws its furs from different-sized areas, and these areas often change in size and shape. The Bureau of Statistics (7) data begins in 1919 and the latest report is for 1951. This report lists the collection for Canada as a whole and also gives the totals for each province. The relative importance of the various species and the provincial distribution of these species is discussed by Butler (1).

From these sources of information it is possible to plot the yearly fur collections for the whole or parts of the country and study the changes from

year to year. Thus it is possible to get a quantitative picture of the yearly and regional changes in fur collections. The chief objection to using such data as an index of population changes is that we do not know that the same proportion of the population is trapped each year. Indeed there is some evidence available which indicates that a greater proportion of the population is trapped during a period of abundance than during a time of population scarcity. Fur values and other economic conditions also have their effect as discussed by Butler (2). In spite of these drawbacks all available information indicates that, when the population size increases or decreases, the fur collection also goes up or down in a relationship which is direct but not proportional.

The questionnaires were begun in 1927 and have been continued in various forms until the present, with the exception of a break in 1932. Much of the information in these questionnaires has been published at various times by Elton and Chitty. The main point in these questionnaires is that observers at various points in the country are asked to state whether the different species were more abundant, less abundant, or show no change from the previous year. With competent observers stationed in the same locality for a long period of time, this method could be quite effective. However, not all observers were competent or even conscientious, so that isolated reports which are at variance with those of the surrounding area should probably not be given too much weight.

The main contrast between the information given by the fur returns and that given by the questionnaires is that one tends to measure the area over which the increase is taking place while the other measures the size of the increase. In discussing data from these two sources, no attempt will be made to correct them back to year of production instead of the year in which the animals were caught or in which the data were compiled. So in this paper the year 1936 refers to the fur collection or questionnaire for the year from June 1935 to May 1936.

Are These Data Cyclic ?

Cole (6) shows that chance data, derived from Tippett's series of random numbers, show a cycle which is similar to tree ring data. From data of this sort, the author concludes that much, if not all cyclic data can arise from similar chance occurrences. This hypothesis introduces some much-needed scepticism into a subject where investigators have been prone to read cycles of various lengths into all kinds of data. There are, however, two main objections to Cole's treatment and conclusions. In the first place, his definition of a peak is open to objection. He attempts to get away from the subjective determination of peaks by the rigid definition that peak is "any year in which the population is greater than in the year preceding and in the year following". This definition is a perfectly good one for theoretical periodicity data or in those rare cases where the cycles are well-marked and perfectly regular. For most actual data where successive values fluctuate at

random around a trend this definition is inadequate. Slight bumps in a cyclic trough, or an exceptional low value during a period of cycle build-up, as well as other chance departures from trend would appear as peaks. This is illustrated in Fig. 1. Since no other objective definition of a peak can be substituted for Cole's, it appears that the subjective choice of the peaks, providing it is followed by appropriate checks, is still the best method. MacKenzie (13) in his study of grouse has also the same opinion. Cole's peaks are made up of three values, the center one being the peak. This means that with irregular data the application of this definition will tend to split it into groups of three. Because there will be some points which will not be contained in these groups of three, the average cycle length will be a little more than three. The more regular the cycle becomes (providing it is longer than three units) the longer the apparent cycle becomes. This is illustrated by applying successive smoothings to Fig. 1.

Fig. 1 is a composite graph prepared from the lynx data to show the types of situations encountered in pelt collection figures. Dealing with this by the ordinary method we find four peaks. The first of these would be placed at

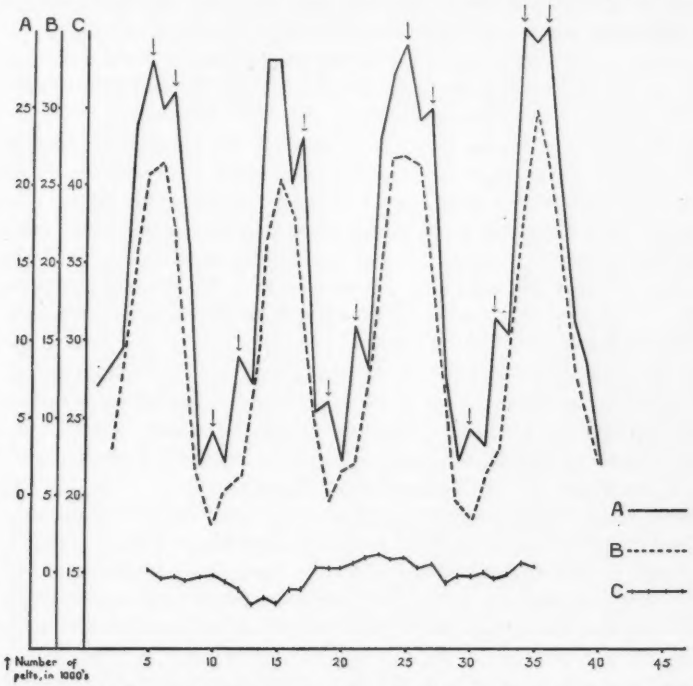

FIG. 1. Composite graph of annual pelt production to illustrate two methods of determining peaks.

A. The original data.
B. The 3-year running average.
C. The 10-year running average.

year 5 if we use the maximum, or at year 6 if we use the trend. The second peak is a split one with equal catches on two succeeding years. The peak year from the trend would be year 15. The next peak is at year 25, and the last peak occurs in year 35 between the twin maxima of the preceding and succeeding years. These four peaks in 40 years give an average cycle length of 10 years.

If we apply Cole's definition of a peak we get peaks at the points marked by arrows. There are 13 such peaks in 40 years giving an average cycle length of 3.08 years. If we use 3- and 10-year running averages on these data it is possible to determine whether there are 3- and 10-year cycles present. These running averages are also illustrated in Fig. 1. It will be seen from this graph that the three-year average smooths out the minor fluctuations and accentuates the 10-year cycle. The four peaks of the 10-year cycle are unchanged but the 13 peaks by Cole's criterion have been reduced to four. The 10-year average, as expected, straightens out the data. If there had been a three-year cycle present in the data as well as the 10-year one, the 10-year average should show peaks at approximately three-year intervals which it does not. Thus with these data the use of Cole's peaks would give an erroneous impression. The data are typical of a 10-year cycle but the application of Cole's criterion of peaks would lead one to interpret the data as a three-year cycle. For longer cycles Cole uses the 'high' peaks and provides a formula for determining the number of these but not their position.

The second objection to Cole's hypothesis is one which he forsees himself, namely, that if peaks occur fortuitously, then various regions should not show a peak the same year. Most workers using Canadian fur return data have postulated that the peaks do occur the same year throughout most of Canada. Butler has shown that there are regional differences in peak years but that these could easily be explained by migration. All the published data are confined to certain regions or specific animals so that a larger series is needed to refute or confirm this synchronism.

In order to test whether the peak populations tend to synchronize throughout Canada, or whether the peaks occur at random, the fur collection area was divided up into 63 sections. These sections were chosen in such a way that they would be as ecologically homogeneous as possible, and that each would contain from one to six Hudson's Bay Company posts. In making these divisions, topographical and vegetation maps were consulted but some compromises had to be made in order to get representative data. These sections were made for forecasting purposes and for assessing the fur potentialities of the country, but they are probably superior to single post returns or electoral divisions in that the grouping of similar data makes the peaks more prominent. Tabulating the peak years for the collections of red fox, lynx, fisher, mink, muskrat, and white fox we get the data contained in Table I. It is obvious from this table that peaks do not occur at random nor do the peaks occur in all sections on the same year. The figures show a definite pattern in their arrangement, fisher, fox, and lynx give one grouping of peaks, mink and

muskrat give a second grouping, and white fox a third. Totalling up the data for each group, we get sufficient numbers to make the pattern evident. For instance, in the group comprising colored fox, fisher, and lynx, we find that no sections experienced peaks in the years 1920 to 1923. In 1924, one section had a peak in fox and four sections had peak collections of lynx, giving a total of five peaks for this year. In 1925 there was a total of 21 peaks with 35 the following year, 28 the next year, and only one in 1928. After this there were three years in which no peaks occurred. In the 35 years there are tabulated 266 peaks for these three species. If the peaks were entirely

TABLE I

FREQUENCY OF PEAK COLLECTIONS OF VARIOUS FURS IN 63 SECTIONS OF CANADA

Year	Red fox	Fisher	Lynx	Group total	Mink	Muskrat	Group total	White fox
1916–17	6	5	16	27	—	—	—	—
1917–18	6	6	3	15	—	—	—	2
1918–19	1	—	—	1	1	—	1	3
1919–20	—	—	—	—	—	—	—	—
1920–21	—	—	—	—	1	5	6	—
1921–22	—	—	—	—	11	17	28	5
1922–23	—	—	—	—	2	7	9	2
1923–24	1	—	4	5	18	4	22	1
1924–25	13	—	8	21	2	1	3	1
1925–26	18	5	12	35	6	—	6	—
1926–27	12	8	8	28	—	—	—	9
1927–28	—	1	—	1	—	—	—	—
1928–29	—	—	—	—	—	—	—	—
1929–30	—	—	—	—	—	1	1	3
1930–31	—	—	—	—	—	1	1	9
1931–32	—	—	1	1	1	25	26	—
1932–33	—	—	2	2	9	10	19	—
1933–34	8	—	4	12	16	1	17	3
1934–35	19	3	2	24	11	1	12	8
1935–36	11	4	6	21	1	—	1	—
1936–37	4	2	3	9	—	—	—	—
1937–38	1	1	—	2	—	1	1	10
1938–39	—	—	—	—	—	1	1	2
1939–40	—	—	—	—	1	22	23	—
1940–41	—	—	—	—	6	10	16	3
1941–42	6	—	—	6	12	6	18	8
1942–43	9	—	2	11	15	—	15	2
1943–44	14	2	4	20	6	—	6	1
1944–45	4	—	3	7	1	—	1	1
1945–46	14	—	5	19	—	—	—	9
1946–47	—	3	—	3	—	—	—	3
1947–48	—	—	1	1	1	3	4	—
1948–49	—	—	—	—	1	19	20	—
1949–50	—	—	—	—	10	4	14	9
1950–51	—	—	6	6	10	2	12	3
Total	147	35	90	272	141	142	283	97
Mean	4.2	1.0	2.6	7.8	4.0	4.1	8.1	2.8
Chi square	281	149	186	449	255	591	793	138

P in all cases $< .0001$

random events then we would expect 7·6 to occur each year. Testing the departure of the observed from the expected, we get a chi square of 469 with a P value of $< .0001$. Similar calculations for individual species and for each grouping of a species, prove beyond a doubt that peak collections are not something that occur at random. This is true even of the white fox which is taken in only 14 sections and has a short (four-year) cycle. Its chi square, while the lowest of the lot, it still significantly large.

While the foregoing tests can be taken as proof that these peaks do not occur at random but are grouped into series of 'good' years, similar tests also show that the hypothesis of a similar peak year for all sections is also unsupportable. Too many peaks occur in years near the orthodox peak to be dismissed as chance errors.

The Nature of Population Cycles

We have shown that peak fur collection numbers are not random occurrences. Instead there is a definite pattern of a number of 'bad' years in which no section has a peak, followed by a number of 'good' years in which many sections experience peaks. The good years begin with only a few sections showing a peak, work up to the point where the maximum number of sections show the peak, and then fall off again to a minimum. The good and bad follow one another in regular sequence, and one set of good years plus the succeeding or preceding set of bad years equals one cycle.

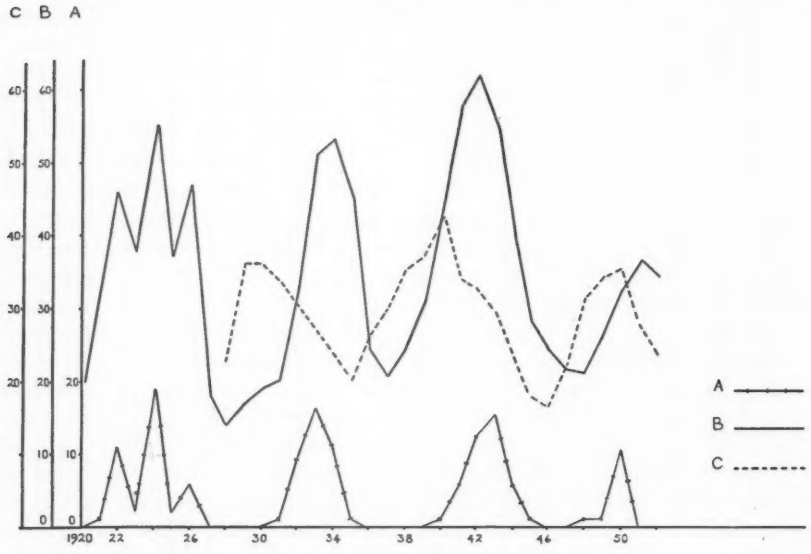

FIG. 2. The mink cycle as determined by three sources of data.
A. The number of sections showing a peak collection.
B. The pelt collections for Canada.
C. The percentage of observers reporting increase.

The classic conception of a cycle is that the increase takes place more or less evenly throughout the whole area in which the cycle is exhibited. This increase continues until all sections reach their peak at more or less the same time, and then begin to fall off. The numerous publications of D. and H. Chitty (3) on the snowshoe rabbit enquiry have shown that in different sections of the country, rabbits begin to increase and also reach their peak at various times. Butler (2) has also shown this to be true for the red fox and has suggested that regional differences are due to migration. From Table I, we get another impression; namely, that the peak year is the one which has the largest number of sections showing a peak. Thus in Group 1 the peaks were in 1917, 1926, 1935, and 1944 with 27, 35, 24, and 20 sections showing peaks in each of these years respectively. The point now arises; do the maximum number of sections show a peak the same year as the fur collection graphs? This may not be, because the collections from each section are not equal, so that peak collections in a few high-producing sections may outbalance peak collections in a larger number of poor-producing sections on succeeding years. In order to obtain information on this point, the fur collection data, and the number of sections showing a peak, were plotted for the six species in Table I. The results for mink are typical and are given in Fig. 2.

The fur collection figures for mink (Fig. 2) show four peaks which occurred in 1924, 1934, 1942, and 1951. Three of the peaks appear perfectly regular although it is probably too early to be sure about the 1951 peak, which is little over half the height of the other peaks. The 1924 peak is very interesting. The main peak is preceded and succeeded by minor peaks which occur in 1922 and 1926. In the same figure, we have shown the number of sections which had peak returns in each respective year. It will be seen that there is a correlation between these two sets of data but the relationship is not perfect. In the second cycle, the peak collection occurred in the largest number of sections in 1933, but the total production did not reach its peak until the following year. The same thing happened in 1950, whereas in 1943 the opposite situation occurred, the peak of total production coming one year before the maximum for number of sections. In the 1924 peak, the two sets of data are in perfect agreement. The reason for the triple peak in the 1922–1926 period is apparent when the geographic distribution of the section peaks is studied. In 1922, peak populations occurred in Manitoba, but most other provinces did not reach their peak until 1924. Ontario, which has the largest population of mink, did not reach its peak until 1926; so in this case it is evident that the lack of synchrony in the state of the population in different parts of the country is the reason for the triple instead of single peak. Thus it is evident that selection of data can, and does, influence the timing and magnitude of the peaks.

Since the triple peak of 1922–1926 is plainly the result of one- and two-year differences in the peak collections of various localities, it is worthwhile examining the other peaks to determine whether they too are made up in a similar,

but not as extreme a manner. We find that in the 1934 peak, all provinces except Alberta and the Northwest Territories had a peak in this year. The Northwest Territories experienced its peak in 1933, and Alberta did not have a peak between those of 1924 and 1942. In 1942, all provinces except the Northwest Territories and Saskatchewan had a peak. These two provinces had their peak in the previous year. The reason that the lack of synchrony does not show in these two peaks is that the provinces which are out of phase do not have a large enough collection to cause a significant deviation in the total Canadian collection.

These data for mink have shown us that peak collections occur as the resultant of simultaneous high collections in most parts of the country. Rarely are all parts of the country exactly in phase; whenever a high-producing section gets out of phase it may produce a secondary peak or even a split peak.

In an effort to find out more about the regional distribution of peaks, the fur collection data for colored fox in the main producing area were divided into two parts; those for the prairie provinces, and those for Ontario and Quebec. The collections from British Columbia and the Northwest Territories, because of their limited production, were not used. The two groups are roughly of equal production value. The data are plotted in Fig. 3. This graph shows a smooth 10-year cycle in the prairie provinces with peak collections in 1925, 1935, and 1944. The data for the Eastern area show peaks in

FIG. 3. The pelt numbers of colored fox taken in the eastern region, prairie region, and the whole of Canada. Ordinate in 1000's.

1926 and 1936, each one year later than the corresponding peak in the western area. In 1944 and 1946 there was a split peak which further analysis reveals is the result of a large southern population in 1944 and peak northern population in 1946. The eastern cycle appears to be one to two years behind the western one. If the graph for eastern production is moved two years to the left, the two polygons show excellent agreement.

The fisher or pekan displays a similar type of cycle. This animal has never been as abundant as the other fur bearers, the peak collections have been between 8000 and 10,000 pelts, and the lows between 2000 and 3000 pelts. Since 1870, there has been a constant decline in production. In spite of the small numbers and the declining trend there is a definite cycle in the pelt production figures. Since 1832 there have been 12 peaks, or one peak every 10 years. The intervals between peaks, beginning with the peak of 1841, have been as follows: 10, 10, 10, 10, 9, 11, 8, 10, 8, 10, 9, and 10 years. A breakdown of the peaks shows that they do not occur in the same year in all sections, being one or two years earlier in Manitoba and British Columbia than in Ontario and Quebec.

The fur production figures for the muskrat of Canada have been published by Elton and Nicholson (11). These data show the same type of 10-year cycle exhibited by other fur bearers, but many of the peaks tend to be less definite. If the data for Canada are split up into regions, the peaks become more definite and the cycle more regular, indicating that the different sections are slightly out of phase. The fur returns for muskrat have been unpredictably changed in the past because of their greater dependence on rainfall and drought. The returns of the last few years have also been affected by conservation schemes, closed seasons, and controlled trapping. Marten shows a similar cycle, but the decrease in catch, and the imposition of closed seasons, have made the cycle hard to trace in recent years. The pelt data for wolves are cyclic, with the coyote showing a definite peak every 10 years, while the timber wolf displays the split peaks characteristic of data made up of several populations which are slightly out of phase.

The classic example of a population cycle is the Canadian lynx because the fur returns for 220 years show a periodicity of great regularity and large amplitude. These data have been quoted by many writers and are effectively summarized by Elton and Nicholson (12). Their report shows that the average cycle length is 9·6 years, and that all regions are not in exact phase. This latter point is illustrated in Fig. 4, where the 1926 peak is split into three of its component parts. This graph shows that Manitoba and Saskatchewan reached their peak in 1924, while Ontario and Quebec did not reach their peak until three years later. British Columbia and Alberta reached their peak in 1925, and Northwest Territories, not included in this group, reached its peak in 1926. So that in this one cycle, the lynx population reached its peak first in the central province, then a year later in the western provinces, and finally two years later it peaked in the eastern provinces. Since such

political boundaries are arbitrary divisions as far as wildlife is concerned, it would be worthwhile to attempt to establish the natural boundaries, and also to find out whether there is a standard sequence to these peaks.

Elton and Nicholson used a more natural grouping and they conclude, "There is no regular line of progression or geographical contouring in the incidence of peaks in different regions that can be easily seen when they are mapped in detail. But the results of a rough method of calculation suggest that there is a tendency for the peak to appear first in Athabasca Basin Region and spread west, north, south, and east, and to appear last in Lakes and Gulf regions." The idea of using the Athabasca Region as the point of origin for peaks was suggested to Elton by Rowan and is also firmly entrenched in the minds of practical fur buyers. Since such a sequence of peaks would be of great importance in cyclical research it is worthy of further study. A clearer picture of these regional differences can be obtained from the questionnaire data which, because of their greater emphasis on area, are better adapted for this purpose than are the collection data.

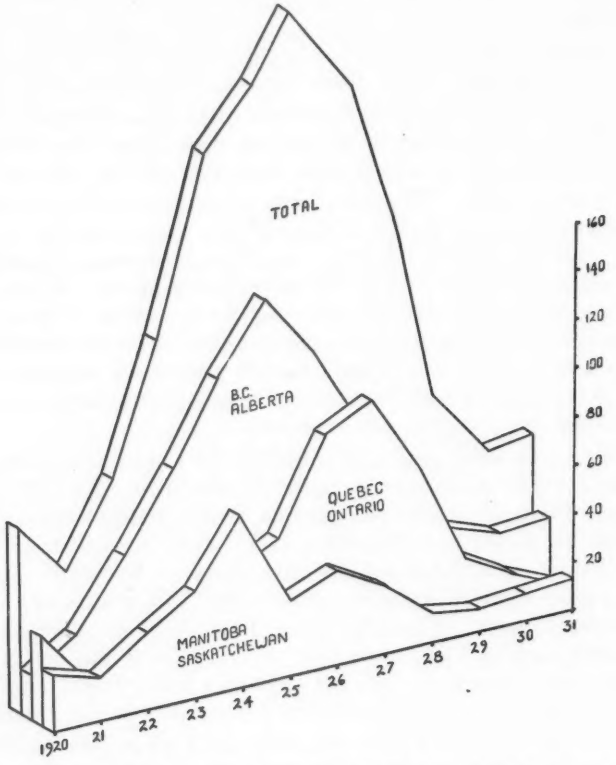

FIG. 4. The pelt numbers of lynx taken from different sections of Canada during one cycle. Ordinate in 100's.

The data from the questionnaires have been plotted to give an annual map of population change for each species. On these maps it is possible to join up points which report the same type of change in population. This method can be employed to produce isophasal lines which delineate regions of population increase from areas of decrease or no change. Treated in this manner the map for any animal for the first year of cyclic increase consists of one or more islands of increase surrounded by large areas of no change or decrease. The next year the islands will often occupy a greater area, then a composite map for the two years can be made to show whether the latest areas of increase are new ones, or whether they have spread out from the old areas.

Applying this mapping technique to the data for snowshoe rabbit we find the cyclic lows were reached in 1927, 1936, and 1945. The following year, in each case, some observers were reporting increases. Each year thereafter a greater proportion of observers reported increases until four to six years later when the maximum percentage of increase was reached. After this there was a short period in which the number of observers reporting decreases kept getting greater until a new low was reached. The period of increase following the 1927 low is not well documented and will be omitted from this discussion. The other two will be examined in some detail to determine whether there is a basic pattern.

The 1937–1939 period of increase began as large areas of increase in Alberta and Manitoba, connected by a narrow bridge across Saskatchewan. In 1933, the bridge expanded to take in most of the province of Saskatchewan. At the same time, the area of increase in Alberta expanded to take in most of the province, while in Manitoba a similar but less extensive increase took place. In this same year two fingers of increase pushed out for some five hundred miles into Ontario. The north finger follows the shore of Hudson Bay while the south finger plunges southward to Lake Superior. In 1939, the north finger regressed with all posts in this area reporting decrease, while the south finger consolidated its gains and expanded its area. Incidentally the regression of the north finger was only temporary because by 1940 increase had again set in and was reported from all over this area. In 1939, the area of increase also spread westward into British Columbia and northward into the Northwest Territories, as well as covering the lower parts of the prairie provinces. In Quebec in 1939, most posts were reporting increase, so that except for a small strip in Ontario, most of the snowshoe rabbit habitat was carrying a larger population than in the previous year. These areas of increase are shown graphically on the map in Fig. 5.

The wave of increase which took place in the years 1946–1949 again began as two small areas in the northern parts of Manitoba and Alberta, which coalesced to produce the configuration shown in Fig. 6 for 1946. It will be seen from this figure that the increase had again begun in the northern parts of the three prairie provinces and in the three succeeding years had gradually covered the whole country. In this period of increase the spread into Ontario was more orderly than was the case in the 1937–1939 period.

The wavelike spread of areas of increase across the country is extremely interesting. It indicates that the first signs of recovery from a cyclic low are seen in the northern parts of Alberta and Manitoba. In succeeding years, reports of increase occur at the fringe of the area reporting increase the previous year, and spread out from this. The easiest biological explanation for these observations would be that as the rabbits increase in numbers they migrate out into the less populated surrounding areas, the direction and the extent of such migrations depending on population pressure and the current suitability of the habitat encountered. Such a theory is contrary to our generally accepted ideas of snowshoe rabbit cycles, but it seems preferable to the alternate hypotheses: 1. That extrinsic factors, such as weather

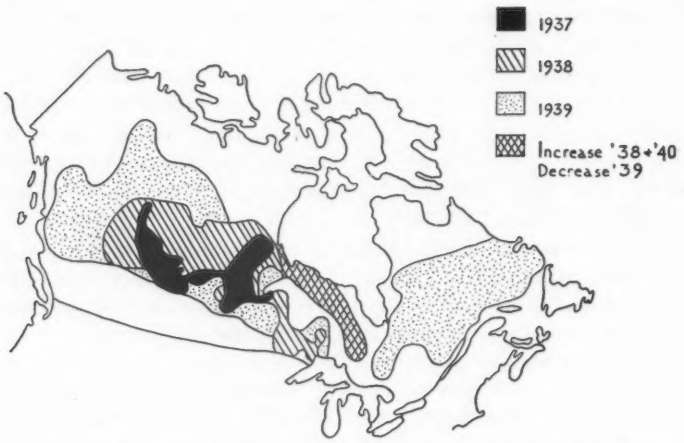

FIG. 5. Isophasal lines delineating areas of Canada in which observers reported increase in the snowshoe rabbit population.

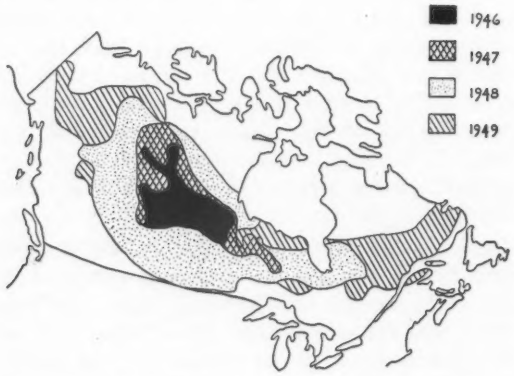

FIG. 6. Isophasal lines delineating areas of Canada in which observers reported increase in the snowshoe rabbit population.

conditions, move in a three-year wave across the country. 2. That intrinsic factors can be communicated from one location to adjacent ones, so that the rabbits can say, "The population to the west of us has increased, we must do the same". The migration theory can certainly be supported from population densities, and from the previously published colored fox data, so it should be borne in mind as a working hypothesis when examining the data.

Turning next to the population highs, we find that in 1933 and 1940, observers are almost unanimous in reporting increase over the previous year. The only exceptions which occur are irregular areas near the Quebec–Ontario provincial boundary, the north shore of the St. Lawrence River, and the extreme northerly part of Manitoba. In the 1950 high, the picture is much the same except that a large part of southern Quebec is reporting decrease. In this high there is also a strip of decreases reported along the Churchill River throughout most of its length in Saskatchewan and Manitoba.

The decrease, which began in 1941, took place first in isolated areas of Manitoba and Alberta and by 1943 covered most of the prairie provinces. In 1944 it had extended to cover most of the country. This followed the same pattern as the years of increase and would fit in with the accepted theory that decreases begin when an optimum population is reached, and that it would take a standard number of years to go from scarcity to this optimum. During the 1950 high, we noted that areas of decrease had appeared along the Churchill River and in southern Quebec. In 1951 these two areas became connected by a broad strip extending across Ontario. This is entirely different from the pattern of decrease noted in the previous period. One possible explanation is that this area is identical with that known to have been subjected to an epidemic in beaver. The cause of the epidemic is believed to have been a tularemia-like organism which would most likely have affected snowshoe rabbits.

Turning next to the questionnaire data for colored fox, we find that the pattern of increase is similar to that shown by the rabbit. The increase begins in the northern parts of the prairie provinces and covers a larger area each year, so that in three to four years the whole country is finally reporting increase. The only exception is in the Hudson Bay and James Bay area. In this section, increases occur which are not connected with the generalized wavelike pattern. For instance in 1945, at a time when most observers were reporting decrease, all coastal points around the Bay were reporting increase. This and similar irregularities are easily explained by assuming that such coastal points are affected by the northern four-year cycle as well as by the 10-year one. If this explanation is accepted then two alternative hypotheses can be suggested. First, that a build-up of the fox population in the north has led to migration, and that this migration has followed the coast in coming southward. Such coastal 'runs' are characteristic of both the white and colored foxes in the arctic regions. The second hypothesis is that the same mysterious influence which causes the fox population in the arctic to increase spills over to the south and affects both coasts of the Bay.

In support of the first hypothesis is the fact that at Moose Factory, on the southern tip of the Bay, the peaks occur in 1917, 1926, 1936, and 1946. While at Attawapiskat, to the north, there are peaks in 1918, 1923, 1926, 1928, 1934, 1938, 1942, 1946, and 1950, indicating the influence of the northern four-year cycle.

The crash phase is extremely short-lived. In 1935 and 1943, small areas of decrease appeared in northern Saskatchewan and by the next year had spread over most of the country.

Mink show a wave of increase similar to that of the snowshoe rabbit except that the boundaries are not as definite. The 1946–1949 period of increase began in northern Manitoba and Saskatchewan. By 1946, it had completely covered these two provinces and spread just over the borders into the adjoining provinces. In 1948, it had spread all the way across northern Ontario and along the Quebec shore of James Bay. To the west, it had spread halfway across Alberta. By 1949, increases were reported all over the country except in the Yukon and northern British Columbia, while in Saskatchewan, where the increase had begun, a few reports of decrease were mapped. The corresponding cycle in muskrat began a year earlier with an area of increase in the same region as the 1946 one for mink with the addition that increase also took place in the Mackenzie Delta. By the next year increase reports covered all of the three prairie provinces and had spread into Ontario and the Northwest Territories. The 1947 map shows no extension of the range, but by the following year, all of the country except British Columbia and the Yukon were reporting increase. By 1949 scattered areas of decrease appeared, indicating that the cycle had reached its peak the previous year. In the preceding period of increase the pattern was different. It began in 1936 with small areas of increase in northern Manitoba, Great Slave Lake region, and the Mackenzie Delta. In the next year, Saskatchewan did not show any reports of increase although the map showed that the area of increase had now spread all across Manitoba and into Ontario. In the two succeeding years, the reports of increase cover the whole country as can be seen in Figs. 18–20 of Elton and Nicholson (11).

One would expect the lynx map to show a strong similarity to the map for snowshoe rabbit, but this is not so. Increases and decreases in this species do not begin at one or two points and then spread across the country. Instead, small areas of increase appear on the map and stay for two or three years without extending their range. The generalized picture is the same as would be observed if there were four or five independent and relatively isolated populations. Whether the wavelike spread of increase was present in lynx before its numbers were reduced cannot be ascertained from existing data. It will be seen that the pelt returns plotted in Fig. 4 indicate a wave which began in Manitoba and Saskatchewan and spread in each direction.

The maps for ermine are very similar to those for lynx, except that the local areas of increase do tend to get larger and often coalesce. There seems to be no similarity of pattern between the increase phase of successive cycles.

This is to be expected since the fur returns do not show a country-wide cycle, but rather a collection of four-year cycles which are not all in phase. Wild mice show a picture which closely parallels that of ermine. Fisher, marten, squirrel, and skunk do not show a wavelike spreading of increase and decrease although the first two species show definite cycles in their pelt returns.

The data for arctic fox have been extensively dealt with by Chitty (4) who concludes that "some regions are out of phase with the rest". There is certainly no indication in these data that reports of increase or decrease form a wave pattern. Of course, with the arctic cycle of only four years' duration, it would be difficult to detect such waves of increase even if they were present.

From the questionnaire data, we conclude that for the three species, snowshoe rabbit, colored fox, and mink, the cycle begins its increase phase in the northern section of the prairie provinces. From this initial area of increase, the reports of increase spread out unevenly in each succeeding year until practically the whole country is covered and the cycle had reached its peak. The decrease phase follows much the same pattern. Muskrat follow the same pattern but deviate from it fairly frequently. The rest of the fur bearers do not follow this pattern but increase more or less independently during the good years. The data do not cover a long enough period so that one may be sure that each cycle brings forth similar patterns or lack of patterns in the species mentioned above. Nor can we be sure that the reason for the lack of pattern is not an artificial one introduced by the fact that lynx, marten, and fisher are rarely seen whereas snowshoe rabbit and fox are easily seen and their population changes quickly noted.

The Relationship Between the Population Peaks in the Various Species

In order to determine whether the peak collections of the different species occur at the same time, Fig. 7 was constructed. In this figure, no attempt was made to designate one year as the peak, but rather the good years, or years when peaks are being experienced in various parts of the country, were recorded. Inspection of this figure shows that the good years for snowshoe rabbit are evenly spaced throughout the 37 years under consideration. The good years for lynx, fox, fisher, coyote, and wolf are closely connected with

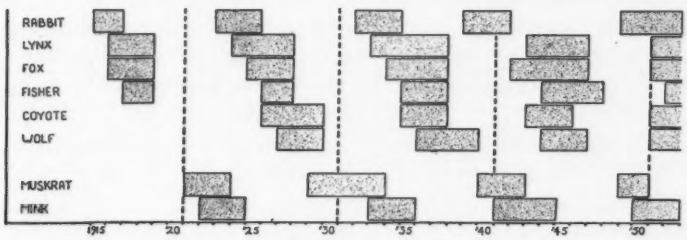

FIG. 7. The relationship between good years in the various species. Good years shown by stippling.

those for rabbit, usually beginning in the middle of the good years for rabbit and continuing for several years after. These animals themselves are also closely associated. The lynx and fox populations increase first and are followed a year or two later by those of fisher, coyote, and wolf. The cycle for muskrat and mink, although of almost the same length, does not seem to be in phase with the other animals. There is a close association both in cycle and habitat between muskrat and mink.

To investigate these relationships, use was made of the same sections as described for Table I. If there is a real association between the population changes of these different species, it should show more clearly in the relative homogeneity of these small areas. Accordingly, colored fox because of its wide distribution was used as the reference animal for the first group of related species. In each section the date of each peak in the fox collection was noted; then the peak years for other species were recorded as the number of years before or number of years after the respective fox peak. This process was repeated for all sections, and the data summarized in Table II. This shows that in most of the sections, the peak collection of lynx pelts, and the peak collection of fox, occurred in the same year. In the remainder, 38 cases out of 54 had a lynx peak one year before the fox peak, while 11 sections had it one year after the fox peak. In the first four peaks, which took place in this 37-year period, the sections are almost evenly divided between the two peaks that occur simultaneously, and the lynx peak, which occurs one year ahead of the fox peak. The fourth cycle shows a different distribution with absolute synchrony in 12 sections out of 21, and with the peaks in other sections scattered from three years before to two years after the fox peak. This last cycle was much weaker in amplitude than the previous ones, and showed no peak in many sections. These data show that the peak populations (using pelt collections as the criteria of the state of the population) of colored fox and lynx do occur at approximately the same time, the most frequent deviation from synchrony being when lynx reach their peak one year ahead of the fox.

Fisher did not occur in as many sections, so only 46 peaks could be compared. In 18 cases the peak came the same year as that of the colored fox, and in

TABLE II

THE RELATIONSHIP BETWEEN THE PEAK COLLECTIONS OF DIFFERENT SPECIES

Peak No.	Lynx peaks in relation to fox peaks							Mink peaks in relation to muskrat peaks						
	Years before				Years after			Years before				Years after		
	3	2	1	0	1	2	3	3	2	1	0	1	2	3	4
1			12	11	3					2	10	12	8	3	2
2		1	13	15	2					1	7	11	16	4	1
3			10	14	4						5	12	16	4	2
4	2	1	3	12	2	1									
Total	2	2	38	52	11	1				3	22	35	40	11	5

24 cases it came one year after the fox peak. Thus it appears that the lynx population responds more quickly to the good years than does that of the fox, and the fisher responds more slowly. This could be anticipated from a knowledge of their breeding habits. The lynx bear young the same year as they are bred, and these young are mature and can breed the following year. Fisher, because of delayed implantation, do not bear young until a year after they are bred, and these young do not produce the next generation of young until they are two or three years old.

Mink and muskrat peaks were treated in a similar manner using muskrat as the reference species. The results (Table II) show that the peak of the mink population usually comes one or two years later than the muskrat peak. In some sections of the country, notably the Mackenzie Delta, it tends to be three or four years later. In only three cases did the mink peak occur before the muskrat one, and in each case it could be attributed to migration of an adjoining population. Mink populations are fairly mobile, as can be deduced from muskrat conservation schemes. When a large population of muskrats is built up on a marsh, a similar increase is seen in the mink population. Many of these mink are not native to the area because the local mink population has been heavily trapped under a predator control system, and tracking studies show many mink tracks leading to the marsh along the water courses.

Discussion

We have shown that the periodic increases in population for any species do not reach their peak simultaneously all over Canada. There are lags of one to three years between the first and the last section to reach a peak. Thus if one postulates an over-all causal factor such as weather, then one has to assume that it also shows lags, and affects different parts of the country at different times. An alternative explanation is that the animal exists at different population densities in various sections of the country. Now if all parts begin to increase at the same time, then the rate of increase in different sections will not be the same, because rate of increase is partly dependent on the initial density of the population. Density in the early stages of cyclic increase acts as a stimulant. It makes sure that all females have a chance to mate and produce young, and also that most of the females will be mated by selected virile males. As a simplified example, let us take an animal which can produce up to eight young per year. Now if we postulate an adult death rate of 50%, then each female would, on an average, have to raise two young to maturity to maintain the population. So if we take two sections of different density we could have the following picture:

Section A: Fall, 100; spring, 50; increase, 50; total, 100.
Section B: Fall, 1000; spring, 500; increase, 500; total, 1000.

Now assume that conditions are right for an increase, then section A will produce four young per female, while Section B, because of its more efficient mating density, will produce six young per female. The increases will now be 100 in A and 1500 in B. So there will be 50 more animals present in

Section A and 1000 more animals present in Section B. The small increase in Section A would probably go unnoticed; it would be a year or so before the population would reach a high enough density to take advantage of the greater mating efficiency and the higher rate of reproduction of a larger initial population. This assumes that the number of young which would be produced and survive is directly proportional to the population present in the earlier years of population increase. After the point of inflection in the sigmoid curve, the opposite condition would hold, owing to intraspecific strife and other factors. This is the type of relationship we would expect in animals which follow the type of increase shown by *Tribolium* but not that which would be expected from animals following the *Drosophila* type of increase. In Section B the population would quickly reach the state where intraspecific strife, and other density-dependent factors would make distant pastures look green, and some migration would take place. It would be interesting to know whether the migrants consist of the cowards and misfits which are driven out, or the more vigorous and venturesome which get out; also whether the migrants come from the fringe of the more densely populated area, or from the center of this area. These points would have a bearing on any scheme which involves migration.

Assuming that migration does take place, then the emigrants from sections of high initial population would settle in surrounding areas of less dense initial population and this would lead to a large increase in this area for two reasons. First, the breeding population has been increased by the immigrants. Second, the breeding population may now be large enough to warrant a larger rate of increase. Under these conditions, migration would act as a synchronizing element. If, because of low initial populations, or for other reasons, one section lags behind the rest, migration into this area will quickly bring it up towards its peak population, so that its peak will occur only one or two years after the rest.

Migration from populations which have been isolated for several generations will tend to bring in polygenes which have been lost by chance or selection in the local population. The crossing of such strains will in many cases give hybrid vigor and rapid increase. The evidence for migration is rather limited as no successful tagging experiments have been carried out. It is known, however, that in the fall of 1942 colored foxes were extremely abundant at York Factory, and relatively scarce at Island Lake 300 miles inland. After freeze-up the fox became exceedingly scarce at York Factory and abundant at Island Lake. Another piece of evidence was uncovered in 1943 when notes were taken on the abundance of fox in a marshy area with few 'travel-ways'. The notes indicated a definite seasonal concentration on certain travel-ways and a general exodus from the area. That fur animals can travel long distances through strange country has been shown by the northward migrations of colored fox into Baffin Land and other arctic regions. Recently the arctic fox has begun to retaliate by making sporadic excursions deep into colored fox territory. In the spring of 1951, a white fox was taken at Cat Lake in Ontario,

and in the following February, one was taken at Russell in Manitoba. Both of these places are almost 400 miles south of the nearest white fox habitat.

On the basis of the foregoing, we can make the following postulates: There are a large number of factors affecting animal populations. Some are favorable and some are unfavorable. By chance alone a large number of favorable independent factors will appear together with a frequency proportional to the total number of independent factors. Thus in the bush country, there are more factors than in the tundra which means that favorable combinations should appear more frequently in the Arctic and this may account for the shorter cycle. Many of these factors will be connected either directly or indirectly with the weather, but it is naive to expect to find a high correlation between any single weather component and animal populations. In the first place, meteorological units are not biological ones. Saturation deficit is more useful than humidity measurements, while an animal does not respond to air temperature as an entity, but in relation to wind, humidity, ground temperature, and sunshine. Also, a spring which favors reproductive success may be counterbalanced by a summer conducive to high mortality. So that any attempt to correlate favorable and unfavorable factors with population changes will have to make use of a composite index, and utilize the method of matrices rather than Pearsonian correlation.

If all factors were independent of one another, the chances of favorable combinations could be worked out on a probability basis, but the factors are not all independent. For instance, reproductive efficiency is probably dependent on population density, so that some sections will respond to favorable factors to a greater degree than will others. Under these circumstances, or when conditions are favorable in some sections and not in others; migration acts as a unifying and synchronizing agent.

While the grouping of peaks into good years and the pattern in the spread of increases point to migration as a causal factor of cycles in some areas, the association between cycles of different species points to an external agency which acts in a somewhat similar manner on many species. The speed and direction of the response to the causal factor depends on the innate qualities of each species and the state of its population at that particular time. There has been much research directed toward the finding of the causal factor, but none of it has been successful. In the planning of future research it is worthwhile looking at some of the more obvious reasons why the research of the past failed to achieve its objective. In the first place, a single causal factor was being sought, rather than a complex of factors. As a result of this viewpoint, the causal factor for the cyclic increase of lynx was given as the increase in snowshoe rabbits. These predator–prey relationships were accepted without much critical study, largely because they seemed logical and partly because there was little precise information available on population changes in prey animals. Accordingly research was directed at finding the reason for the prey cycle on the thesis that it governed the predator ones, whereas there is equally good evidence that many of the causal factors in the rabbit cycle are also

causal factors in the predator cycles. That the two types of cycles are not always intimately connected can be seen from the fact that in some areas rabbits will be increasing while lynx are dying off, and that arctic fox will decrease while lemming are still at their peak.

The cooperative experiment to find out whether rabbits produce more young on the upsurge of a cycle than they do on the die-off is a typical example of the hunt for a single causal factor, as are the disease investigations of MacLulich, who sought the cause of die-off as a single factor disease. This last investigation had another reason for failure, if the conclusions drawn in this paper are correct. Namely, the location at which the work was undertaken. This investigation took place in Ontario where snowshoe rabbits never reach a fraction of the population density that they do in Alberta. MacLulich was dealing with cycles with little amplitude, and consequently found differences in phase in populations a few miles apart. When the population did go up, he had no way of knowing whether they were local animals that were increasing, or immigrants. Any future investigations should take place in sections where high population densities are reached, should check on the influence of migration, and should look for a complex of causal factors instead of one single factor.

Acknowledgments

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THE PTILINAL ARMATURE OF FLIES (DIPTERA, SCHIZOPHORA)[1]

By E. H. Strickland[2]

Abstract

The ptilinum of schizophoran flies usually is described as a simple protrusible bladder, the function of which is that of bursting the puparium immediately prior to the escape of the adult. A few observers had, however, recorded that it may be provided with scalelike projections. An examination of the ptilina of approximately 150 species of flies, representing 40 families, indicates that these scales are a characteristic of all schizophoran families. Many of these are described and their structure is compared. Nearly all of the extreme variations can be correlated with similar structures on the proboscis of a conopid. In the majority of families, they are very small and usually conform rather closely with four major types which merge into each other. Exceptions to this general rule occur in certain genera in widely separated families which include Ephydridae, Tetanoceridae, Anthomyidae, and Tachinidae. The action of the ptilinum in recently emerged Calliphoridae indicates that its armature of scales may render it an effective digging organ and that these flies employ it energetically for this purpose. With it, *Cyanomyia cadaverina* Desv. can remove the smoked surface from a block of plaster of Paris. Extreme scale development occurs on the ptilina of those genera of Tachinidae in which the adults escape from subterranean earthen cells of phalaenid hosts. Their heavy sclerotization may be completed in the pupa several weeks before the adult normally emerges. They are relatively about ten times as long as are those of many related genera. Among field-captured specimens many show that a high percentage of the scales on the upper and lower surfaces of the ptilinum have their apices missing, indicating extensive abrasion as the fly scraped its way through the consolidated earthen cell of the host. From their armature, it appears that the buccal bladders at the anterior base of the rostrum may, in certain families, assist in effecting an escape.

Introduction

The first published record regarding the ptilinum of schizophorous Diptera appears to have been that of Réaumur (11) in 1738. He correctly ascribed to it the universal function of assisting the adult to escape from the puparium by its intermittent expansion and contraction.

Though several subsequent writers have discussed the structure of the ptilinum of individual species of flies, few have suggested that it is capable of performing anything other than that of rupturing the puparium by simple pressure. It is, in fact, most frequently referred to simply as a "protrusible bladder" or words to that effect.

In 1911, Knab (7) drew attention to the fact that, as early as in 1863, Becher recorded that the ptilina of *Musca*, *Calliphora*, *Lucilia*, and *Sarcophaga* spp. are beset with "lozenge-shaped scales". Despite this, subsequent investigators do not appear to have attached much importance to a possible

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use for these structures. Knab, however, also quoted Reising to the effect that newly emerged Tachinidae use their ptilina to "push obstacles out of the way" after their escape from the puparia, though he did not elaborate upon how this is accomplished.

In 1916 Graham-Smith (2) stated that recently emerged *Calliphora erythrocephala* distend their ptilina in order to "obtain a purchase against neighboring particles", thus suggesting an appreciation of a possible use for the scales. Jobling (6), who carefully figured the ptilinal scales of *Glossina palpalis*, states, simply, that the pulsation of the ptilinum helps the fly to pass through earth and decaying humus.

Laing (9), in a valuable contribution on the development and the function of the calliphorid ptilinum, paid particular attention to its musculature. She described and illustrated the scales, which she termed "spinules", but did not discuss their possible function. Neither the word "scale" nor "spinule" describes the varying forms in which these structures occur in different Schizophora. In the interests of brevity, the earlier designation, "scale", is here adopted to cover all types.

How universal these are on ptilina and the extent to which their structure may vary are matters which do not appear to have been given much attention. Imms (5), in his *Textbook of Entomology*, states that the "ptilinum is roughened owing to the presence of minute scales or spines of various forms". Hendel (3), in the *Handbuch der Zoologie*, describes them as *often* beset with "chitinige Dörnchen". Other recent textbooks to which we have referred still give a reader the impression that the ptilinum is a simple smooth-walled bladder. In the most recent treatise on the ptilinum which we have seen, Atkins (1) mentions their occurrence in *Drosophila melanogaster* Mg. but does not suggest that they might be put to any use. They are, admittedly, much smaller and more indefinite in this family than is usual and, in comparison with others, can probably be correctly described as "degenerate".

Our attention was drawn to them in a series of the tachinid *Bonnetia compta* Fall. which, some years previously, had been reared from the subterranean cells of a phalaenid, *Agrotis orthogonia* Morr. One specimen had failed to retract its ptilinum before it was killed and "pinned". The powerful black spurlike scales on this could not fail to attact the attention of an unaided eye (Fig. 7).

This led to an examination of the ptilina of representatives of all schizophoran families available in the University of Alberta collection. Practically every one of nearly one hundred and fifty species, representing about 40 families, possessed scales. These, normally, are so closely crowded together over the entire ptilinal surface that they overlap. Exceptions occurred in certain of the ephydrids and tetanocerids in a few species of which they were completely suppressed. In the only coelopid available to us the small, almost circular, scales are rather widely scattered.

We feel, therefore, justified in concluding that the ptilinum of all Schizophora normally is densely clothed with an armature of scales.

The Origin of Ptilinal Scales

Künckel d'Herculais (8) demonstrated, in 1875, that the ptilinum is identical in structure with the rest of the body integument. Laing, in studying its larval and pupal development in *Calliphora*, confirmed this but showed that, subsequent to emergence, the underlying epidermis, and much of the chitin itself, disappears.

An examination of the epicranium reveals that scales frequently extend, from the ptilinum, over much of its surface. Of considerable interest is the fact that in certain families, of which Conopidae is a good example, they extend, chiefly in the connective membrane, right to the apex of the proboscis. Along its course, they change in structure in such a manner that they conform with nearly all of the variations which we have found on the ptilina of the different schizophoran families that we have examined (Fig. 1).

Furthermore, similar scales are of widespread occurrence on the integument, particularly in association with connective membrane, throughout the Arthropoda. Their structure embraces a wide variation of which, it appears, the villi on the wings of many insects are an example. Laing and others have established that the ptilinal scales are merely excrescences of the cuticle.

In addition to the cuticular scales, the ptilina of representatives of every family examined possess some 14 or more simple setae scattered over their surface. Each can be seen to arise through a circular pore in the cuticle from the underlying epidermis of recently emerged flies. In certain tachinid species, there appeared to be at least 50 setae. Whether or not these have a sensory function was not established. Usually they do not project upwards for much more than the average length of individual scales. Rarely, as in a helomyzid, *Suilla nemorum* (Fig. 31), though they are of normal size over the greater part of the ptilinum, they gradually lengthen towards its dorsal margin, near which they are produced into a row of elongate pigmented bristles.

Material and Methods

Practically all of the material examined consisted of pinned specimens from the University collection. Recently emerged calliphorids and tachinids, examined before the ptilinum had been permanently withdrawn, revealed no external structures which differed from dried material.

For the preparation of microscope slides, heads were removed and were boiled for a minute or two in 10% potassium hydroxide. Following this treatment it is sometimes possible to extrude the ptilinum by forcing water or air into the head through a fine glass tube inserted in the occipital foramen. This method, however, is not suited to microscopic examination and, owing to the extreme thinness of the "mature" ptilinum, it is not often successful. It is, however, readily accomplished in recently emerged and untreated adults. In such cases, permanent mounts can be made by employing the technique used in inflating larvae (Fig. 6).

Dissection of potassium hydroxide treated heads is a practical alternative in preparing microscope slide mounts. This was most satisfactorily accomplished

in a drop of water on the slide on which the permanent mount was to be made. The eyes were first removed with a scalpel fashioned from a razor blade chip. The proboscis was then cut off, as was the posterior half of the head. After living adults had been observed attempting to escape through glass it was realized that the proboscis should have been retained. Its "buccal bladders" frequently are armed with powerful scales which, in the species which possess them, probably play some part in effecting an escape. What now remains is the front of the head, less the eyes, from the vertex to the oral margin. If it is turned over, the relative volume of the retracted ptilinum can be observed, whether it hangs down medially (Fig. 11) or is simply "bunched up" behind the suture.

On account of its hemispherical shape, the ptilinum cannot be flattened out and it must be cut or torn in order that much of its surface may be examined.

What is left of the epicranium is, therefore, turned face up once more, the frons is bisected by a vertical cut to the frontal suture, and, unless the lower ends of the latter extend to the genal angles, they are continued by vertical cuts to the severed margin of the epicranium. At this time, it is advisable to flood the specimen with 96% alcohol since this reduces, but rarely entirely overcomes, the tendency for the ptilinum to double back on itself when traction is released. The severed halves of the *frons* are now pulled apart resulting in the dorsal half of the ptilinum tearing along its median line to its apex. Two further floodings with 96% alcohol suffice to remove sufficient water for the preparation to be mounted by the addition of a drop of diaphane just before the last of the alcohol has evaporated (Fig. 2).

This technique is satisfactory for preparing the majority of ptilina for examination though it can be approximated only in the case of very small species. Unfortunately, the ptilina of a few species are so extremely fragile that they tear into small pieces under the most careful manipulation. In these cases, fragments only can be examined.

Variations in the Size and the Structure of Ptilina

In the majority of families, particularly among the Acalyptrata, the ptilina are moderately uniform in size and, when distended, they occupy about the same depth from front to back as does the head itself. Those of Conopidae, despite the fact that the frontal suture is unusually short and extends downward to less than one-third the distance from the bases of the antennae to the genal angles, are voluminous and represent the most "rugged" type of ptilinum which we encountered. Their texture is reminiscent of chamois leather. We could not expel them through the suture with occipitally applied pressure and they were too wrinkled in all of our preparations for us to be able to measure their dimensions with any accuracy. Their size was unexpected since, on other characteristics, these flies are usually considered to have closer affinities with the aschizan Syrphidae than has any other schizophoran family.

Dissections of several species of Syrphidae failed to reveal any structures which could, by any similarities, be homologized with a ptilinum, a frontal suture or a frontal lunule. Künckel D'Herculais (8) asserted that, while escaping from their puparia, Syrphidae strongly inflate their frontal region, and cause a small bladder to appear. He stated, however, that all traces of this disappear before the adult is ready to fly. It is certain that, in the syrphid species which we examined, there were no suggestions of ptilinal scales in the sparse connective membrane of this area. It is most unlikely that these would disappear completely after the emergence of the adult had they ever been present. The nearest approach to them were very small short comblike scales on the membrances of the antennal fossae. Becker, according to Knab, denied a ptilinal function in Syrphidae while Laing failed to demonstrate ptilinal muscles in this area.

In other families, with the exception of certain Tetanoceridae, a few Ephydridae and Hippoboscidae, the frontal suture extends nearly, but rarely quite, to the genal angles. In most of the Tetanoceridae the suture is horizontal and lies almost entirely above the antennae. It bends downwards, slightly at the most, at its ends. In some genera, however, it is perfectly normal and the ptilinum is fully developed whereas, in the majority, it is reduced to a mere ribbon which, though armed with an unusual type of flattened scale (Fig. 28), must, surely, be nonfunctional. Attempts to inflate this with occipital pressure merely caused it to bulge outward slightly. In the genus *Sepedon*, the narrow ptilinal area is as heavily sclerotized as is the rest of the epicranium; it is a flat exposed sclerite which is completely devoid of scales (Fig. 10).

In the majority of Ephydridae, despite the greatly swollen face, the ptilinum is normal in development and in armature. In a small group, of which *Ochthera* and *Parydra* are examples, the suture resembles that of the Tetanoceridae, though the ptilinum is voluminous and is armed with a very unusual type of scale (Fig. 25).

Hippoboscidae possess narrow ribbonlike ptilina, which, in this respect, resemble those of some Tetanoceridae. Their scales are, however, of a very different type (Fig. 50).

The greatest relative ptilinal size was encountered in a helomyzid, *Anorostoma currani* Garr. (Fig. 11), though that of *Gasterophilus intestinalis* de G. is almost as large. In many species examined, the retracted ptilinum lies in a crumpled arc-shaped mass immediately behind the suture. In *A. currani*, however, it assumes somewhat the shape of a mushroom of which the stout "stem" is free and projects downwards between the antennae almost to the oral margin. Intermediate stages between these extremes are not uncommon.

Since all of the ptilina here discussed were boiled in 10% potassium hydroxide before examination, they consisted only of such of the cuticle as had been retained at the time of capture. Laing (9) indicates that the underlying epithelium is never robust and that it, with its ptilinal muscles, degenerates soon after the emergence of the adult. In *Calliphora erythrocephala* she finds that the cuticle itself undergoes a marked dissolution. Three

days after emergence it has been reduced in thickness from 25 μ to 5 μ, while, by the 15th day, only the finest layer which connects the bases of the scales remains.

We do not know the individual ages of many of the pinned specimens which we examined but were impressed by the unusual thickness of the cuticle in the ptilina of all Conopidae in comparison with its extreme fragility at the other end of the series, as represented by Tachinidae. Here, it may be almost invisible and is so thin that it is almost impossible to flatten it on a slide without dismembering it. This reduction is even more pronounced in the anthomyid genus *Fannia*, in all species of which it appears to be reduced to the extremely fine layer of cuticulin from which the scales arise.

In individual species of many of the families which were examined, scales, resembling those on the ptilinum, extend from it to additional areas of the epicranium. These occur most frequently on the frons where they may extend to the vertex on either side of the ocellar triangle. They differ from those on the ptilinum chiefly by their tendency to fuse together into compound rods.

Observations made on recently emerged *Cyanomyia cadaverina* and *Phormia regina* during their attempts to penetrate glass with their ptilina proved that, in these flies, the frons functions with the ptilinum at this time. It retains its flexibility and ability to expand until a normal escape has been effected, after which it is contracted longitudinally and becomes sclerotized as part of the inflexible epicranium. Such, however, is not the case in an agromyzid, *Agromyza angulata* Lw. (Fig. 9). In this and other related species, the transparent ptilinum is armed with unusually powerful black scales. These extend, without change in disposition or structure, on to the permanently flexible and transparent frons and reach the vertex on either side of the heavily sclerotized ocellar triangle. It is evident that, in these flies, the two areas constitute a single functional unit of very unusual dimensions.

Scales may also extend from the ptilinum to the frontal lunule and thence down the face as a narrow band between the antennae. This, frequently, widens below the fossae of the latter to cover much of the area between them and the oral margin. In several genera in which the frontal suture does not attain the genal angles, additional scales flare out from its ends and cover much of the genae themselves.

Particularly among many of the smaller species of Acalyptrata, an independent development of scales so heavily arms the rostral membranes that they have every appearance of serving some function. They are particularly well developed on the so-called "buccal bladders" (Fig. 9, *d*). Though these are almost unarmed in Calliphoridae (Fig. 4) they are occasionally pressed by these flies against, and forced in a downward direction over, a glass obstruction which the fly is attempting to penetrate.

Variations in the Structure of Ptilinal Scales

By far the most prevalent types of scales encountered in the species of the 40 families which were examined look, in side view, much like rose thorns (Fig. 1). From above, however, these scales appear as flattened blades of which the outline varies sufficiently for them to be likened to three major types. These can be roughly described as resembling a wheat, barley, or oat grain, respectively.

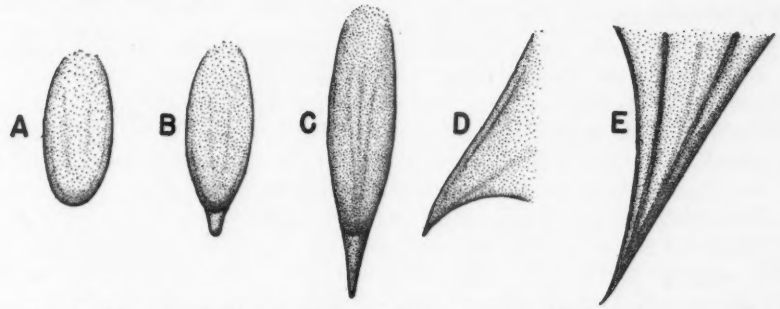

FIG. 1. Normal ptilinal scales (diagrammatic). A, B, and C upper views to types 1, 2, and 3, respectively. D. Side-view of an average 1-3 type. E. Type 4, which varies much in length and degree of attenuation; this type is frequently confined to the median areas of the ptilinum.

Type 1

Approximately twice as long as wide, with a rounded apex.

Type 2

Resembling 1, but the apex is pointed to a varying degree from an obtuse angle through a stout tapered spine to an elongate bristle.

Type 3

Three to five times as long as wide, apex acute and occasionally produced into a spine of varying length or diameter.

In all of these types, the base tends to contract, but it may be parallel-sided or, less frequently, flaring.

Without exception, the apices of all of these types of scales are directed upwards and backwards, i.e. towards the vertex, on the upper half of the ptilinum, and downwards and backwards on its lower half. The change in direction occurs along a transverse belt, of which the width includes about half-a-dozen scales. This "equatorial" area passes through or just below the ptilinal apex (Fig. 6). At the apex itself, the scales are usually reduced in size, are vertical, and somewhat mammiform in appearance. Laterally in this belt they tend to be directed outwards. As will be realized later, during a discussion of their function, this disposition is well suited to their purpose.

Type 4

Any of the above types may merge through intermediate forms into conical structures the sides of which are reinforced by about six deeply pigmented

"rods". Usually this type, which is not often encountered in the "earlier" acalyptrate families, is interspersed with the more normal armature only on certain areas of the ptilinum, which are more frequently median than otherwise. In only a dryomyzid species were the majority of scales of this type. Typically, they are only slightly diverted from a vertical position on whatever part of the ptilinum they occur. It appears, however, that the greatly enlarged scales which are developed (Figs. 48 and 49) on the "working" surfaces of certain tachinid ptilina have been derived from this type of scale. These are numerous and are semirecumbent.

In color, scales vary from unpigmented through light yellow to dark brown, or through gray to opaque black. No reason for the second variation in pigmentation was deduced, though in certain species, there was slight evidence that the grayish pigmentation increased with the age of the individuals. Tachinid scales, if greatly enlarged, are always of the very dark-brown color which is associated with powerful sclerotization on other parts of the body, such as at the tips of the mandibles. Around the periphery and near the apex of these ptilina the scales are not enlarged and may be entirely unpigmented. This suggests that in these flies, these relatively immense "working" scales possess an unusual ability to withstand abrasion and that, whenever this color predominates, it is associated with this characteristic.

For purposes of comparison with a number of other types of scale, it is assumed that type 1 or, possibly, type 2, represents the basic pattern from which, through a variety of modifications, other types, which less frequently occur on the ptilina of different flies, have been derived. In so doing, it is realized that there may be little justification in assuming one type to be more "primitive" for ptilina than any other. Its occurrence is, however, by far the most widespread and it is very rarely entirely replaced. Furthermore, approximations to this type can be found in the connective membrane, or other structures, of very diverse representatives of Arthropoda. Those on the cheliceral shields of the tick *Amblyomma americana* Koch. are almost identical with it though they are more recumbent. Similar scales occur in the connective membrane associated with the maxillae of cockroaches and between the tarsal joints of certain grasshoppers. In the beetle *Harpalus caliginosus* Fabr. many maxillary scales resemble type 2, while some approach type 4.

An examination of the entire ptilinum of all species of flies reveals some variation in the outline and relative size of the scales on different areas of its surface. In many, for example, they are smaller and more blunt with a tendency to fuse longitudinally in the downward extensions of the frontal suture; in others they are more strongly developed medially than they are laterally, while in all the reversal of direction at the apex is, normally, accompanied by a shortening in length of the individual scales and a tendency for a higher percentage of type 4 scales to occur here than elsewhere. Unless such variations are marked, the scales are described as "uniform". Where illustrations have been made, individuals which are of the average size and shape have been selected unless otherwise stated.

Action of the Ptilinum

The action of the ptilina was observed in two species of Calliphoridae; *Cyanomyia* (*Cyanomyopsis*) *cadaverina* R.D., and *Phormia regina* Mg. Puparia were placed in cells consisting of short lengths of glass tubing, of which the inner walls were lined with fine emery paper in order to provide a purchase for the ungues and the dorsocentral bristles. The puparia were held in place, at about half their length from the tops of the cells, with plugs of cotton. The cells were closed above either with clear cover glasses or with plaster of Paris caps of which the lower sides were either smoked or painted black. All covers were held in place with adhesive tape.

Laing (9) describes and illustrates the internal points of attachment of important ptilino-oesophageal muscles along the anterior portion (equatorial region) of the ptilinum. Over this the scales are small and, near its center, are vertical. As already stated, all scales are directed towards the vertex above this region and towards the oral margin below it. When an escaping calliphorid attempts to penetrate glass, the ptilinum, normally, is partly inflated and pressed firmly against the glass with this area withdrawn. This brings the centers of the upper and lower surfaces of the ptilinum together along its infolded margins. A more powerful inflation follows, during which the muscles gradually relax. This relaxation causes the entire upper and lower surfaces of the ptilinum to scarify the obstruction as the tips of the opposing scales are forced apart. Thus, the "working" surfaces of the ptilinum are confined chiefly to the upper and lower median areas. Fig. 3 illustrates the ptilinum of a calliphorid which was inflated before the muscles had disintegrated. It shows a partial withdrawal of the "equatorial region" as a result of a slight contraction of these muscles. In life, at the inception of its withdrawal, five points of muscular attachment are plainly seen along its length as small temporary depressions. In this species, this region is slightly below the morphological apex of the ptilinum whereas in the majority of flies it appears to pass through it.

As can be seen in the photograph (Fig. 4), the partial inflation of the ptilinum is accompanied by a great distension of the buccal bladders. In Calliphoridae these are armed only with relatively weak villuslike scales. They were, however, occasionally brought against the glass with the result that the final inflation of the ptilinum forced them downwards over its surface. This action appeared to be more accidental than purposeful. The shape of the cell, however,—a cylinder with a flat smooth top—is unnatural. Observations made upon species in which the bladders are as heavily armed as is the ptilinum itself may reveal, under more normal conditions, a definite employment of these structures in assisting the escape of the fly.

In an unobstructed emergence, the ptilinum undergoes a series of pulsations for a period of up to about ten minutes before it is permanently withdrawn. It would appear that these pulsations may be essential for the removal of small particles of foreign matter which become lodged between the scales

(Fig. 7). When, however, emergence is obstructed, ptilinal activity may persist intermittently for several hours and it ceases entirely only when the weakened fly approaches the point of death from exhaustion.

The activities of a *C. cadaverina* which was watched continuously for the greater part of a day exemplify, in general, those of a number of specimens which have been under observation. This burst its puparium at some time between 9.00 a.m. and 9.30 a.m. As soon as it made contact with the glass it explored its surface with its fully distended ptilinum. The apex was then withdrawn and the "scarifying" activity was initiated. This was repeated over and over again. In the early stages of expansion minor, apparently exploratory, pulsations suggested an attempt to engage the scales in something that would give a purchase. After about two hours of fruitless endeavor, the glass surface was seen rather suddenly to be bathed in a copious liquid. Michl, as quoted by Knab (7), states that occasionally *Muscina stabulans* will rupture its ptilinum in its efforts to penetrate paper. Nothing so drastic was observed in *C. cadaverina* or in *P. regina*. Later observations, made upon *regina*, showed that this fluid is liberated through the pseudotracheae. In one fly, three large drops were seen in rapid succession to cover the labellae. Extreme activity caused this liquid to flow over the entire head, including the ptilinum, and thence to the obstruction. Only about 50% of the flies under observation employed this liquid and usually only after an hour or so of fruitless endeavor, though one did so only four minutes after it reached the glass. No attempt was seen to place the liquid directly on the obstruction. Its liberation in no way reduced subsequent ptilinal activity though its employment was not observed to occur more than once during lengthy attempts to escape.

After approximately three hours of constant activity, the fly appeared to suffer from temporary exhaustion. During a half-hour rest, the only continuous movement was a slight and regular pulsation, at exactly one-second

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FIG. 2. Muscid (*Musca domestica* L.) ptilinum exposed by technique described. Some tearing has occurred in flattening, a defect that is less liable to occur with more "rugged" types. FIG. 3. Calliphorid (*Phormia regina* Mg.) head, with ptilinum partially inflated to show a slight retraction of the apex as a result of muscular contraction. Prior to an attempt to penetrate on obstacle the apex is completely withdrawn, thus bringing together the centers of the upper and lower ptilinal surfaces. The photograph is of an artificially inflated, and dried, ptilinum, which is somewhat less completely dilated than it may be in life. FIG. 4. Calliphorid (*Phormia regina* Mg.) head with buccal bladders distended. Preparation as above. FIG. 5. Smoked surface of plaster of Paris ×16 after an emerging *Calliphora cadaverina* R.D. has attempted to penetrate it with the aid of its ptilinum. The structure of the plaster probably has been weakened during smoking since similar scarifying did not occur when *Phormia regina* Mg. attempted to penetrate plaster painted with India ink. FIG. 6. Tachinid (*Gonia* sp.) ptilinum artificially inflated and dried. This specimen, dissected from a mature pupa, shows that the scales are fully sclerotized before the adult escapes from the puparium. A narrow "equatorial band" of reduced and vertical scales passes through the ptilinal apex. FIG. 7. Portion of the ptilinum of a tachinid (*Bonnetia compta* Fall.), killed as soon as it emerged from its host's pupation cell, showing particles of sand lodged among the enlarged scales.

All photographs taken by the courtesy of Prof. Brian Hocking.

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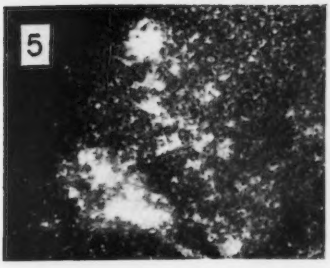
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intervals, of the apex of the frontal lunule below the almost completely withdrawn ptilinum. This was accompanied by a gradual lateral contraction of the frons followed by a rather sudden relaxation at 15 sec. intervals. Activity was now resumed with increased intensity. At times, a portion of the ptilinum would enter a small crevice between the tube and the cover glass. This resulted in what can be described only as "frantic" struggling, followed, invariably, by a careful re-exploration of the rest of the surface with the fully distended ptilinum, of which the apex was now expanded, prior to the resumption of a major effort to escape. These efforts usually lasted for about 30 to 45 min., each followed by somewhat longer periods of almost complete inactivity. During the afternoon, activity became steadily less violent, while the ptilinum assumed an increasingly opaque yellowish coloration. This stage was accompanied by lateral contractions of the hitherto relaxed frons which, temporarily, reduced it to about its normal diameter. By 5 p.m., i.e. about eight hours after emergence, the epicranium showed definite signs of sclerotization and the "leathery" ptilinum appeared to be incapable of further distension. This contrasted with the ptilina of adults which made a normal escape. In all of these, the ptilinum was readily distended, up to at least three days after emergence, by the simple expedient of squeezing the abdomen.

One inexplicable variation in this procedure occurred in a male *P. regina*. In every fly it was seen that the blood, when first forced into the ptilinum, is clear. After about a half-hour of strenuous activity there is an increasing abundance of what appear to be small aggregates of cells suspended in it. These we assume to be haemocytes, possibly with the function of fat body. With the pulsation of the ptilinum they can be seen, passing from the median ventral region of the head through the buccal bladders, to and from the ptilinal cavity. In this specimen, however, they were accompanied by bubbles of "gas". These could be seen, through the still transparent thorax, to be welling upwards along its dorsal wall. During periods of reduced activity those still in the thorax moved with a pulsating motion which, apparently, was induced by the heartbeat. The "gas" was so abundant that it almost completely replaced the blood in the ptilinum and filled the anterior end of the thorax to such an extent that cell aggregates could be observed, high but not dry, held to its wall by a film of blood. Since only an anterior view of the fly was possible the origin of this gas could not be ascertained. Within half an hour of our having first observed these bubbles, the thorax became too opaque for further observation and the ptilinum was permanently withdrawn. In this connection it may be recalled that Réaumur is reported to have stated that the ptilinum is inflated with gas. Possibly he was not so universally incorrect as subsequent observers have assumed. All of this activity, including pigmentation, had occurred in less than two hours from the time that the fly emerged from its puparium. Other flies, which fail to escape, remain unpigmented for at least 24 hr. Four hours after emergence this fly was liberated. It was fully pigmented and normally active though it failed to expand its wings.

The Penetrating Capability of Ptilinal Scales

At the time of writing there has, unfortunately, been no opportunity to ascertain the "digging" power of ptilina in which the scales are abnormally large. Fig. 49 shows the relatively enormous scales on the ptilinum of a tachinid of which the adult normally escapes from the earthen pupation cell of a phalaenid moth. This, surely, must be capable of penetrating far more resistant obstacles than is possible to the majority of flies. In August, 1952, Mr. Jacobson, of the Dominion Entomological Laboratory at Lethbridge, very kindly provided us with a number of pupae of *Agrotis orthogonia* Morr. which contained the puparia of a species of *Gonia*. This tachinid is univoltine and we have failed to break its pupal diapause in the laboratory.

Experiments in which calliphorids were obstructed by plaster of Paris while escaping from their puparia indicated that *C. cadaverina* was capable of making some impression on this material with its ptilinum. Fig. 5 is a photograph of the abrasion which, apparently, was produced by a *cadaverina* in its attempts to penetrate plaster the surface of which had been smoked by holding it over burning xylol. It is probable that the consistency of the plaster had been somewhat broken down by heat during the smoking since, though several of the smoked surfaces showed definite signs of abrasion, nothing comparable could be observed when the plaster was painted with India ink.

From the table of the relative sizes of ptilinal scales in different flies, page 291, it will be seen that those of *cadaverina* are rather below the average but, from the systematic manner in which it "scarifies" an obstruction with them, we are of the opinion that the marked variation in their size in different flies may be an important factor in determining the locations in which individual species can pupate with prospects of successful adult emergence.

Variations in the Structure of Scales on a Conopid Proboscis

Although, with minor variations, the scales on the majority of ptilina are very similar in structure, one or two remarkable exceptions were encountered. These appeared to bear little relationship with the more usual types. It was, therefore, of particular interest to find that, on the proboscis of the conopid, *Zenilla furcillata* Will. (Fig. 8), there is a progressive modification from the "normal" type of ptilinal scale to several of the others which, occasionally, predominate on the ptilina in certain families.

Immediately ventrad of the upturned oral margin a frontal view reveals two large membranous areas, one on either side of the sclerotized clypeus. These, when dilated, constitute the buccal bladders. In Conopidae, the entire rostral membrane is clothed with scales. Over much of the basoanterior region, i.e. on either side of the clypeus, these are of the normal type 2 of many ptilina and are directed downward. Laterally, along the margin of the oral orifice, they turn outward and develop short black tubercles along what is now their ventral side. Meanwhile, the "body" of the scale becomes progressively more narrow and its dorsal side more indefinite until it resembles a comblike structure with from 3 to 12 downwardly directed teeth. This type

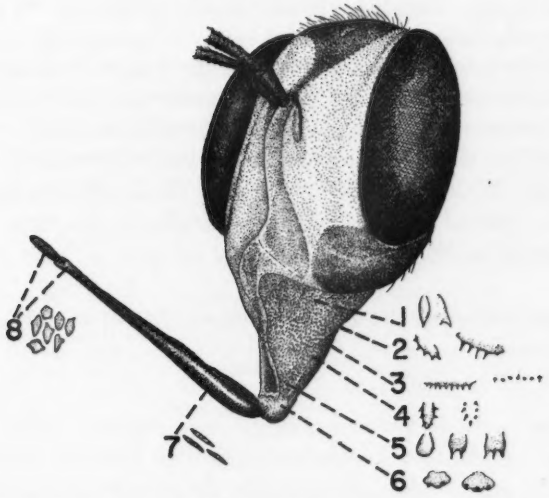

FIG. 8. Conopid (*P. furcillata* Will.) head showing transition of normal ptilinal-like types of scales on proboscis membranes to unusual types found on ptilina of certain families. 1. Normal type 2 scales. 2 and 3. Transition comblike scales degenerating to "villi" posteriorly as on certain ephydrid ptilina. 4. Degeneration of scales to villi in a manner not observed on any ptilinum. 5. Scales with widened or split apices resembling those on chamaemyid ptilina. 6. Reduction of split apices to tubercles as seen on ptilinal apices of certain Agromyzidae. 7. Narrowed tuberculate scales resembling those near genae of chamaemyid ptilina. 8. Flattened scales resembling somewhat the ptilinar scales of certain Tetanoceridae.

of scale covers most of the posterior side of the rostrum but, near its lower end, the "body" frequently disappears completely leaving only a row of minute black spots the majority of which bear a central, very short to rather elongate, unpigmented spine. Similar comblike scales or their residua of black spots constitute the only type of armature present on the ptilina of certain Ephydridae (Fig. 25).

More anteriorly, many scales develop black spurs along both sides and the disappearance of the "body" in these cases leaves a residuum of small clusters of black spiniform spots. Similar structures have not been observed on the ptilina of any flies.

Immediately adjacent to the clypeus the scales tend to sink to the general membrane level and to become more polygonal owing to the widening of the apex. Near the lower end of the rostrum the apex frequently splits into two or more fairly elongate spurs. A similar modification is found among the ptilinal scales in Chamaemyidae (Fig. 26). At the junction with the haustellum they are very irregularly polygonal and are usually armed with from two to four short upwardly directed tubercles. Similar scales occur near the apex of the ptilinum in certain agromyzid species (Fig. 21).

The labrum is unarmed but the rest of the haustellum bears very narrow elongate scales each with a single series of small black spiniform tubercles

throughout its length. Similar structures were observed only on the ptilinum of a chamaemyid, where they were confined to the genal ends of the suture. Near the apex of the haustellum, as well as on the upper surface of the labellae, these scales widen out once more into apparently flattened polygonal structures which, however, in side view are seen to bulge outward in the center. Somewhat similar scales occur on the ptilina of many Tetanoceridae (Fig. 28).

In one species of Conopidae, *Myopa vicaria* Walk., the labellar scales are circular with a central short spur or spine similar in structure to those on coelopid ptilina (Fig. 30).

Comparisons Between the Ptilinal Scales of Schizophoran Families

Conopidae

General

In all species examined the frontal suture is very short and narrow (Fig. 8). It extends downwards only to about one-third the distance from the base of antennae to the genal angles. Despite this, the ptilinum is voluminous and is more "rugged" in texture than is that of representatives of any other family which we have examined. The scales are very closely crowded and they overlap over the greater part of the ptilinum.

Conops fronto Will. Scales type 1. Normal; unpigmented.

Physocephala furcillata Will. Fig. 12. Scales very deeply pigmented with brown. They are so crowded near the center of the ptilinum that this area appears to be completely black under the binoculars and it contrasts strongly with the cream color of the bordering epicranium. Laterally, they are type 1, but medially, particularly on the lower half, the base develops bluntly pointed processes which give their outline somewhat the appearance of holly leaves when viewed from above. The lateral view, however, retains its normal rose-thorn outline. The unpigmented short ptilinal setae are unusually conspicuous since each arises from a transparent pore which, at first glance, resembles a pinprick in the otherwise blackened ptilinum.

Physocephala texana Will. Closely resembles *P. furcillata* though the scales are light yellow in color.

Myopa vicaria Walk. Fig. 13. Scales type 3, with apex produced into a moderately long, highly refractive spine; unpigmented. Under a low microscopic magnification the entire ptilinum appears to be "hairy" owing to the high refraction of both sides of these spines.

Zodion fulvifrons Say. Scales type 2, with a short blunt apex; unpigmented.

Otitidae (Ortalidae)

General

The frontal suture reaches the genal angles, but the ptilinum within its lower ends is partly sclerotized and the scales fuse together into heavily sclerotized rods.

Seioptera vibrans L. Fig. 14. Scales chiefly type 1, varying to 2; unpigmented. They extend to the face between the antennae and tend to fuse to elongate ridges at its center though they separate into a patch of individual scales just above the oral margin.

Chrysomyza demandata Fall. Very similar to *S. vibrans* though the scales are confined to the ptilinum.

Eurycephalomyia myopaeformis V.R. (*Tetanops aldrichi* Hend.). Fig. 15. Scales type 2, apices of those on lower half of the ptilinum produced. Though this species pupates below ground, the scales are small and very light yellow in color.

TRUPANEIDAE (TRYPETIDAE)

General

In three genera examined, the ptilinum is voluminous and it hangs as a median fold internally to about opposite the end of the second antennal joint. The suture is evanescent before the genal angles and its contained ptilinum is lightly sclerotized from about level with the apices of the antennae. Scales are chiefly type 3, with a slightly produced apex. They are closely crowded and overlap.

Epochra canadensis Lw. The very light yellow scales are uniform over the entire ptilinal surface.

Rhagoletis pomonella Walsh. As above with somewhat wider scales.

Eurosta solidaginis Ft. (Fig. 16) and *E. comma* Wd. Scales as above except that they are distinctly yellow and, surrounding the ptilinal apex, where as is usual they are mammiform and reduced in size, there is a wide band of flat scales which are widely flared basally. Their apices are frequently divided into two or three rather shorter spines. The scales on this area impart to it a most unusual "imbricated" appearance. In so far as we are aware, all flies of this genus escape, as adults, from stem-galls.

PALLOPTERIDAE

Palloptera jucunda Lw. Scales type 2, apex stoutly tapering; unpigmented to very light yellow. They continue medially on the face to below the antennal fossae where they fuse to form rods. The membranes of the fossae, also, are rather copiously armed with type 2 scales above but, below the antennae, they merge through a mammiform to a horizontal type somewhat resembling those on the conopid proboscis (3, Fig. 8).

LONCHAEIDAE

Lonchea polita Say. Fig. 17. The majority of the smoky-black scales are type 1 or 2. Medially, on the lower half of the ptilinum, they are transformed to type 4. The suture extends to the genal angles where the contained ptilinum remains membranous though, here, the scales are united to form long rods on which, from near the apex of each component scale, arises a long black bristle. A narrow band of scales extends on the face from the lunule to the oral margin. Only between the antennae do they fuse to form rodlike structures. The membranes of the antennal fossae possess a few transverse scales. The buccal bladders are densely clothed with a mammiform type with an elongate dark-brown central bristle.

TANYPEZIDAE

Tanypeza luteipennis K. & S. Fig. 18. The majority of the light yellow scales are type 2 with an acuminate apex. On the lower half of the ptilinum their bases flare. Scales extend to the vertex as simple rods whereas, on the face, they extend to the oral margin as individual, though connected, scales.

CALOBATIDAE

Cnodacophora nasoni Cr. Normal type 1 to 2 light-yellow scales over the ptilinum but fuse in the downward extensions of the suture, which terminate shortly below the antennal fossae.

MICROPEZIDAE

Micropeza lineata V.D. Despite the unusual shape of the head, the ptilinum is normal and extends to the genal angles. The unpigmented scales are chiefly type 1 but, near the equatorial region, many are widened and resemble somewhat the bracts of a pine cone.

PIOPHILIDAE

Piophila affinis Mah. Scales type 1, rather longer than in related families; almost unpigmented.

SEPSIDAE

General

The ptilinum is voluminous and somewhat pendulous when withdrawn.

Nemopoda cylindrica Fab. Scales type 1 to 2. The latter tend to be produced into a refractive apex resembling that of *Myopa vicaria*, Fig. 2; very lightly yellow pigmented. On the face, a narrow band of hardly modified scales extends to the oral margin.

Themira putris L. Fig. 19. The dark-brown to gray scales are not very uniform, chiefly type 1 but elongate towards the upper end of the ptilinum with a tendency to fuse to compound rods. Above the antennae they are bractlike. Compound rods similar to those on the ptilinum extend on the face to the oral margin.

LAUXANIIDAE (SAPROMYZIDAE)

Camptoprosopella borealis Shew. Scales type 2, apex hardly produced; almost unpigmented. Ptilinum of normal size though the suture extends only about halfway to the genal angles.

Minietta lupulina Fab. Scales vary from type 3 with an acuminate apex to a type 4 which closely resembles that of a dryomyzid, Fig. 32. All are "delicate" and are rather poorly defined; faintly pigmented with brown. The suture does not extend to below the lower margins of the antennal fossae and the ptilinum is unusually narrow. Its brown setae are stout and prominent.

DROSOPHILIDAE

General

The ptilinum is relatively small and the scales are very poorly developed in comparison with related families. With the possible exception of *D. funebris*, it would appear that the weakly sclerotized irregular areas which represent the scales over the greater part of the ptilinum cannot perform any useful function.

Drosophila funebris Fab. The light-yellow scales are moderately well developed. The majority are of type 1, tending to become club-shaped near the genal angles. This species pupates below ground.

Drosophila melanogaster Mg. Fig. 20. Over the greater part of the ptilinum the outline of the scales is poorly defined, though each appears to terminate in a fine spine. Laterally and near its upper margin they are more regularly conical with an acuminate apex while in the downward extensions of the suture they are irregularly club-shaped with a tendency to fuse near the genal angles.

Drosophila buscki Coq. The scales over the entire surface of the small ptilinum are even more fragile and irregular than they are in *melanogaster*. They resemble weakly developed examples of the type found on the ptilina of certain Tetanoceridae, Fig. 28.

ASTEIIDAE

Asteia multipunctata Sab. Scales type 2, widened basally and with a long tapering apex; delicate and unpigmented. Unlike *Drosophila*, they are well-developed and are similar over the entire surface of the ptilinum. Almost identical scales heavily invest the antennae but are not present elsewhere on the epicranium.

OPOMYZIDAE

Anthomyza tenuis Lw. Scales type 2, similar to *Asteia* but rather wider and less clearly defined. Those on the antennae are spiniform.

AGROMYZIDAE

General

A number of the species examined possess a large ptilinum with heavily sclerotized scales. Since these are better developed on the median area than they are laterally it would appear that they may perform a definite function.

Though the majority of species are believed to be leaf-miners the fact that several pupate below ground may have some bearing on the fact that these small flies possess scales which are far better developed than are those of their close relatives.

A feature which appears to be unique to this family is the complete suppression of sclerotization on the frons in a number of species. This is associated with the development of powerful separate scales on this area. Among the species examined, this tendency was not particularly apparent in *Agromyza perpusilla* Mg. but was increasingly so in *A. artemesiae* Kalt., *A. pusilla* Mg., *A. neptis* Lw., and *A. dubitata* Mall. It reached its climax in *A. angulata* Lw. (1, Fig. 9) and *Disygomyza lateralis* (2, Fig. 9). In these two species, the frons is completely membranous and lacks a distinct demarkation between it and the ptilinum which therefore extends as a perfectly transparent membrane to the vertex on either side of the ocellar triangle which, in addition

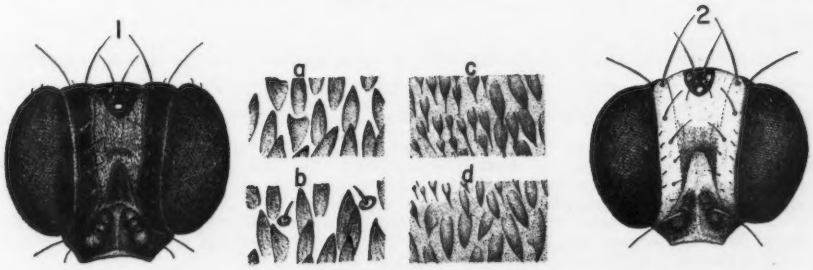

FIG. 9. Agromyzidae. 1. *A. angulata* Lw. "Ptilinum" extends as a heavily scaled transparent membrane to the vertex. *a.* Scales on the permanently flexible frons. *b.* Scales and setae on the ptilinum. *c.* Scales on the partly transparent but rigid lunule. *d.* Scales on the partly pigmented buccal bladders. All scales are drawn at a magnification of ×500. 2. *D. lateralis* Mg. (*A. coquilletti* Mall.) Black ptilinal scales extend about half way on the transparent and flexible frons to the ocellar triangle. Reduced and unpigmented scales extend almost to the vertex. Though the parafrontia are unpigmented, they are rigidly sclerotized.

to the parafrontia, acquires its normal sclerotization. In *A. angulata* the ptilinal scales extend, without interruption or change in structure, to the vertex whereas in *D. lateralis* they are much reduced in size and lose their pigmentation at a short distance below the ocellar triangle. In both species the dorsal and lateral boundaries of this membranous area are sharply defined and it is evident that it is, functionally, part of the ptilinum.

Agromyza dubitata Mall. (Fig. 21) and *A. neptis* Lw. Both appear to be very similar with heavily sclerotized blackish-gray scales chiefly of type 2, with tendencies towards type 4. A rather lightly pigmented band covers the "equatorial" region of the ptilinum. Where this passes through its apex the scales are much widened basally and have their apices separated into from two to about four short blunt teeth. They thus closely resemble the scales on the conopid proboscis (6, Fig. 8).

Surrounding this area a number of scales are asymmetrical. The frons is partially sclerotized and its scales are fused into compound rods.

Agromyza angulata Lw. Fig. 22. Scales type 3, with tendencies towards type 4, unusually large medially where they are dark gray to almost black. Those on the completely transparent frons are black and of type 3 (1*a*, Fig. 9). There is a slight reduction in their size

near the vertex. The sclerotized ocellar triangle and the parafrontia carry a normal complement of delicate "villi". The large sclerotized frontal lunule (1*c*, Fig. 9) and the antennal joints are heavily armed with black, type 2, scales, while the centrally pigmented buccal bladders, also, are armed with brown type 2 scales, which are almost as well-developed centrally as are those of the ptilinum. Around the edges of the bladders they are reduced to a weak villiform type (1*d*, Fig. 9). Webster and Parks (14) state that this species attacks timothy grass leaves and pupates in the mine that it has produced.

Disygomyza lateralis Fab. Scales type 1 over most of the ptilinum but type 2 in the lateral sutural grooves. Several of the latter have their apices divided. The frons is as completely membranous as it is in *A. angulata*, but the black scales lose their pigment before, and become evanescent at about, the apex of the ocellar triangle with the result that a transparent membrane extends on either side of it to the vertex. In untreated examples of this species the head, with the exception of the triangle and the antennae, is white when viewed from in front. Just above the frontal suture, however, it is marked with very fine grayish irregular longitudinal lines which are, in fact, the dark "ptilinal" scales of this area (2, Fig. 9). Webster and Parks (14) record this species under the name *Agromyza coquilletti* Mall. as mining in the basal leaves of volunteer wheat and oats.

Pseudonoapomyza atra Mg. Scales of normal size, chiefly type 1; uniform and gray in color. Frons only partly sclerotized as in *A. dubitata*.

Phytagromyza orbitalis Mel. Scales of normal size, chiefly type 1; brownish-gray. Consistencies of the frons and scales resemble these characters in *A. dubitata*.

PHYLLOMYZIDAE

General

The ptilina and their armatures vary so much in the few species supposedly belonging to this family which are available to us that some doubt arises as to whether they are correctly associated.

Pholeomyia indecora Lw. The scales are rather large, chiefly of type 2 with an acuminate apex; rather dark gray-brown; heavily sclerotized. The ptilinum is voluminous and is pendulous medially when withdrawn. It reaches to about the apices of the antennae. The partly sclerotized frons resembles that of *Agromyza dubitata*.

Anthomyza sp. Scales small, poorly defined, many somewhat truncate-cone to club-shaped with an elongate terminal bristle; very lightly sclerotized and unpigmented. Frons partly sclerotized; surface longitudinally wrinkled.

Desmometopa latipes Meig. Scales very similar to *Anthomyza*, as is the frons though it is more heavily sclerotized.

Desmomyza confusa Curr. Scales well-defined, type 2, uniform; majority light brown-yellow with scattered individuals more deeply stained. All are heavily sclerotized. The frons is very heavily sclerotized throughout and is sharply differentiated from the ptilinum.

CHLOROPIDAE

General

Nothing unusual was observed in any of the species examined. The scales are small, poorly developed, uniform type 2 with apex produced; practically unpigmented.

Thaumatomyia glabra Mg. Scales not contracted basally. Though this fly pupates below ground the scales appear to be very weak.

Meromyza americana Fit. Almost identical with *T. glabra*.

Oscinella frit L. Fig. 23. Scales somewhat more contracted basally than in preceding species and relatively larger. Similar scales cover the buccal bladders but, posteriorly, on the rostrum, they become comblike.

EPHYDRIDAE

General

Despite the greatly swollen face of many species, the ptilinum and its armature usually is normal. It extends within the frontal suture to the genal angles.

In one group, however, comprising *Ochthera*, *Parydra*, and some others, neither the ptilinum itself nor its armature in any way resembles that of other

families which we have examined. Taxonomically, they do not appear to be closely associated with the other genera which are included in this family.

Ephydra millbrae Jones. Scales normal type 3 with a tendency to flare basally and with the apex somewhat produced; dark gray. The equatorial region shows as an unusually light transverse band.

Paracoenia turbida Curr. Fig. 24. Scales type 3 terminating in a long, stout, spine. Heavily sclerotized, dark brown. Immediately above the antennae they are much dilated and many are nearly circular behind the apical spine.

Ochthera mantis DeG. Fig. 25. The frontal suture is transverse and is not produced downward laterally. In this respect it resembles members of the family Tetanoceridae. In dried specimens it appears to continue downward, rather close to the inner eye margins, before bending inwards to the genal angles. This furrow, however, is completely sclerotized and inflexible. The ptilinum terminates abruptly at its upper end in a series of rodlike sclerotized folds.

The somewhat voluminous and intensely wrinkled ptilinum is armed on its dorsal third with transversely elongate comblike gray scales, armed on their upper margin with about twenty powerful dark-brown teeth. They somewhat resemble the comb scales on the posterior base of the conopid proboscis (3, Fig. 8). Over this area, the wrinkling of the ptilinum is, for the greater part, transverse. At the equatorial region, wrinkling is more irregular and the scales, as such, disappear though the teeth occasionally persist as a few scattered black tubercles on the ridges of the ptilinal folds. This parallels the disappearance of the similar scales on the conopid proboscis. The ventral half of the ptilinum is most irregularly and intensely wrinkled in a manner reminiscent of the convolutions of a mammalian brain surface. Very rarely do the summits of the folds bear one or two black tubercles. The entire ptilinum is lightly stained with brown.

Parydra nitida Cr. The disposition and structure of the ptilinum closely resemble that of *O. mantis* but it is completely devoid of scales. The summits of the wrinkles are very finely crenulate.

Borboridae

Borborus equinus Fall. Scales light yellow, chiefly a wide type 2, except in the downward extensions of the suture where, in contrast, they become unusually elongate with a tendency to fuse together to form yellow sclerotized rods.

Clusiidae

Clusiodes melanostoma Lw. Scales type 2, base not contracted, apex produced to a long bristle; unpigmented and delicate. Resemble Chloropidae.

Chamaemyidae

Chamaemyia polystigma Mg. Fig. 26. The moderate sized, light brown scales vary in shape on different areas of the ptilinum. Nowhere have they a regular "grain"-shaped outline. Over the greater part of the ptilinum the apex is bifurcate or trifurcate; laterally on the dorsal surface they are reduced in size and are mammiform with a long apical bristle. Ventrally, the lateral scales are enlarged and fuse together to form elongate structures of which the component scales retain their spinelike apices. At the genal ends of the suture they are reduced to spiniform rods resembling those on the haustellum of a conopid (7, Fig. 8). On the median area above the antennae the apical bristle is reduced or absent and the apices of many scales are widened and obliquely truncate.

Tetanoceridae (Sciomyzidae)

General

Earlier observers have drawn attention to the fact that representatives of this family, generally speaking, possess only a transverse frontal suture above the antennae which lacks lateral extensions towards the genal angles. There is, however, a wide variation in this character among the representatives of different genera. In *Melina* the suture almost reaches the genal angles and it contains a normally voluminous ptilinum. In *Tetanocera* it terminates just below the lower margins of the antennal bases and the ptilinum is moderately voluminous. It appears to be capable of normal evagination. The scales,

for the greater part, are modified into irregular plates with a stout, frequently elongate, central spur. In *Limnia* and *Hadroneura*, however, the suture terminates above the antennal bases and is only slightly arcuate above them.

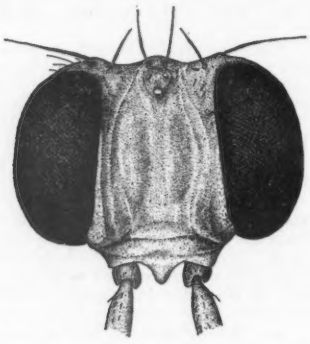

FIG. 10. Tetanoceridae = Sciomyzidae. (*S. fuscipennis* Lw.) All species of the genus *Sepedon* which have been examined are devoid of a ptilinum. It is replaced by a rigid transverse sclerite above the biarcuate frontal lunule.

Here, the ptilinum is ribbonlike and is only slightly invaginated. Water pressure, applied through the foramen, hardly bulges it outwards. The scales vary in development. The most extreme modification is exemplified in flies of the genus *Sepedon* (Fig. 10). These possess *no* ptilinum. The permanently exposed "ptilinal" area is smooth, rigid, and flat. It holds the narrow transverse suture open as a sclerotized area connecting the frons with the transverse frontal lunule.

Melina (*Pherbellia*) *schoenheri* Fall. Fig. 27. Scales normal, chiefly type 1 to 3, merging to type 4; almost unpigmented. Though the downward extensions of the suture reach the genal angles, the ptilinum is flexible for only about one-third of these extensions below the bases of the antennae. This is, however, not uncommon in other families. Type 3 scales, which tend to fuse near the genal angles, clothe the sclerotized portions.

Melina nana Fall. is similar but the scales are brown.

Tetanocera valida Lw. Fig. 28. Though the suture extends only to the center of the antennal fossae the ptilinum is moderately voluminous and is armed over most of its surface with irregular unpigmented scales of which the bases, when viewed from above, resemble rather closely placed pieces of a jigsaw puzzle. In side view the majority appear to be subconical while the apices of those near the median margins of the ptilinum are produced into stout semirecumbent spurs.

Limnia saratogensis Fit. Fig. 29. The subarcuate transverse suture terminates laterally above the level of the antennal bases. Its ribbonlike ptilinum is armed with mammiform scales which are produced to moderately long central spines. Dorsally these unpigmented scales are wide basally and they overlap; ventrally their bases tend to be quadrate. Over the narrow equatorial region they are of the jigsaw puzzle type as in *T. valida*, though they become comblike laterally.

Hedroneura rufa Panz. The ptilinum is even narrower than is that of *Limnia* and it appears to be armed throughout with jigsaw puzzle type of scales which are very weak and perfectly flat over the greater part of its area.

Sepedon armipes Lw. and *S. pacifica* Cr. In common with three other available species (Fig. 10) of this genus, the permanently exposed ptilinal area is as heavily sclerotized as are the neighboring areas of the epicranium. It is smooth and shining. By transmitted light, subtransverse darkened areas are visible, but there is no suggestion of scales.

CHRYOMYIDAE

Trixoscellis fumipennis Mall. Scales, type 1; unpigmented. The suture nearly reaches the genal angles. Nothing abnormal.

PSILIDAE

Pseudopsila perpolita Joh. Scales chiefly narrow type 3 with the apex of many produced into a fine spine; unpigmented. The ptilinum extends to the genal angles. Nothing abnormal, though the ptilinum is rather larger than in related families.

COELOPIDAE

Coelopa frigida Fab. Fig. 30. All of the scales are nearly circular and mammiform; they are only slightly deflected from a vertical position above and below the ptilinar apex. They are moderate closely crowded except in the area across the "equatorial" region where they are separated by more than their own diameters.

HELOMYZIDAE

General

Although in *Suilla* and *Neoleria* the ptilinum is rather more voluminous than usual, it assumes a transverse position within the head when retracted. In *Anorostoma* and *Helomyza*, it is relatively enormous and it extends, as a median fold, almost to the oral margin (Fig. 11 *a*). A distended *Anorostoma*

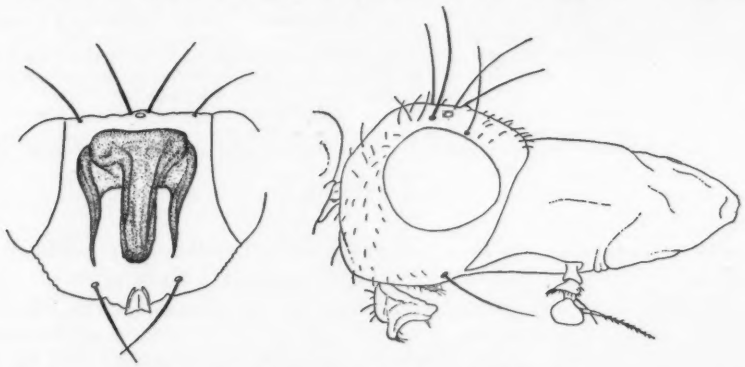

FIG. 11. Helomyzidae. (*A. currani* Garr.) An example of the largest ptilinum seen on any fly. Other species in this family equal it in size but this is rarely approached in those of other families. As seen from behind after removal of the back of the head and almost fully extended from a potassium hydroxide treated head.

ptilinum is conical and, even when slightly collapsed, is nearly one and a half times the length of the head (Fig. 11 *b*). The finely wrinked frons rarely bears traces of scales. They usually are well-developed on the face, particularly between the antennal sockets.

Suilla nemorum Mg. Fig. 31. Scales, type 2 tending to fuse near the upper end of the ptilinum; almost unpigmented. Similar fused scales occur on the lunule and extend between the antennae to below their bases. The most remarkable feature of the ptilinum is its setae. Over the greater part of its surface they are colorless and of a normal size but they gradually increase in length and pigmentation towards its upper margin near which they are transformed into a row of about six excessively long bristles which are about five times the length of the average scale.

Neoleria fuscolinea Garr. Resembles *S. nemorum* but with less tendency for scale fusion while the dorsal setae are less than half as long.

Anorostoma marginata Lw., *A. currani* Garr. and *Helomyza serrata* L. Apart from the unusual size of the ptilinum there is nothing abnormal. Scales, type 1 tending to become somewhat truncate apically and to fuse together near the upper ptilinal margin; almost unpigmented.

DRYOMYZIDAE

Neuroctena anilis Fall. Fig. 32. Nearly all of the scales are type 4, with a bristlelike apex sharply differentiated; rather uniform structurally over the greater part of the ptilinum though, around its periphery, they tend to revert to type 2 with a long apical bristle. The frontal suture extends to only a short distance below the antennal fossae but the ptilinum is of normal volume.

CORDYLURIDAE AND SCOPEUMATIDAE (SCATOPHAGIDAE)

General

In both families the frontal suture extends at least to the vibrissae and is filled to its extremities with flexible unsclerotized ptilinum. The scales are uniform, chiefly type 3 with a tendency to approach type 4 medially on the ptilinum. The frons is armed, at the most with very weak scales, they may, however, be well-developed on the frontal lunule.

Cordilura confusa Lw. Scales medially brownish-black, laterally yellow becoming pigmentless at the extremities of the suture. The frontal lunule is heavily armed with a mammiform type of scale.

Achaetella varipes Walk. Scales longer and more tapered than in *Cordilura*. The suture is longer and the lunule is completely bare.

Scopeuma stercorarium L. Resembles *Cordilura* with more crowded scales which are lightly pigmented on the upper third of the ptilinum in marked contrast with the dark gray-black scales which cover the rest of it. In addition to lunular scales, the antennal fossae of some specimens possess remarkably strongly developed scales on the antennal fossae membranes. These resemble types 5 and 6 of the conopid proboscis.

Scopeuma furcatum Say. resembles *stercorarium* but all scales are uniform yellow in color.

ANTHOMYIDAE

General

Species representing two or more genera of each of the major subfamilies, which have very varied larval habits, were examined. Three types of scales were observed, the most unusual being confined to the genus *Fannia* (Fig. 33). Ptilina in some subfamilies are uniformly more fragile than they are in others. Typically, the frontal suture reaches or exceeds the vibrissae but in the majority of species the ptilinum is sclerotized from about half the distance between them and the antennal fossae. Scales invariably extend to the vertex as composite rods. True scales were not seen on the face, but the buccal bladders, usually are weakly armed.

(Phaoninae). *Bigotomyia houghi* Stn. The fragile ptilinum is voluminous and pendant medically when withdrawn. The scales are type 2 to 3 with tendencies to approach type 4. They are evidently heavily sclerotized and are of an almost opaque brown color. In two specimens examined the apices of several scales were missing which suggests that the fly escaped from a subterranean puparium. We have, unfortunately, no information regarding the biology of this species.

Phaonia basiseta Mall. Resembles *houghi* though the scales are smaller and less heavily sclerotized. No sign of abrasion of the apices was observed.

(Mydaeinae). *Helina barpana* V.d.W. and *Mydaea discimana* Mall. Scales rather longer than in Phaoninae with more of type 4. These predominate on the lower half of the *discimana* ptilinum; brown-black in color but apparently not very heavily sclerotized.

(Hydrotaeinae). *Ophyra leucostoma* Wd. Scales shorter than in Phaoninae and rarely overlapping, predominantly type 4 medially; not heavily sclerotized; light brown. The scales on the frons and lunule are unusually powerful; reduced in size, they also extend on the face to the oral margin. The buccal bladders are densely clothed with strong mammiform, centrally spined, scales.

(Fanniinae). *Fannia scalaris* Fab., *serena* Mg., *postica* Stn., *canicularis* L., *spathiophora* Mall., and *kowarzi* Verr. Fig. 33. The scales of all are practically identical and are quite

unlike those of any other anthomyid genus examined. Over the greater part of the ptilina they are of a compound "comb" type with from about three to eight elongate spiniform teeth. On the upper median area of the ptilinum they break down to singly spiniform scales. The color varied in different species from light yellow to gray.

Demarcation between the ptilinum and the lunule is indefinite, but the former folds downward for a short distance below the latter and, here, it is sparingly wrinkled longitudinally and is completely devoid of scales. The ptilinum is extremely fragile and it breaks into fragments too readily to permit its being flattened. It is, however, sclerotized in the suture from about opposite the antennal bases, below which point the scales form continuous spiniform rods.

Azelia gibbera Mg. Though this genus is included in the Fanniinae, the scales are normal type 1 to 2, lightly pigmented brown but not heavily sclerotized. The ptilinum is more voluminous and stronger than in *Fannia*. In two specimens examined there were faint lateral ptilinal stains resembling those of *Hylemyia antiqua*, Fig. 36.

(Lispinae). *Lispe salina* Ald. Scales all appear to be type 2 and tend to fuse together. They are very heavily sclerotized and crowded.

Lispe nasoni Stein. Fig. 34. The heavily sclerotized median scales are all of a type 4 which approaches the shape of the enlarged median scales of certain Tachinidae. All, however, remain small and no sign of abrasion to their apices was observed in the specimens that were examined.

Incidentally, it was observed that these flies possess extremely large prestomal teeth, a characteristic shared by all genera of Coenosiinae and, to a less extent, Limnophorinae. Hobby (4) has recorded that a number of European Cordyluridae and Anthomyidae are predaceous on various flies, and Perron and Lafrance (10) recently observed adults of *Coenosia tigrina* F., one of the species recorded by Hobby, feeding on adults of the onion maggot in Quebec. From the development of the teeth on the proboscides of every species that we have examined in these three subfamilies, it would appear that all are capable of predatory activities.

(Limnophorinae). *Lispocephala alma pallipalpis* Zett. Fig. 35. Over the greater part of the ptilinum the scales are of type 2 to 3, with or without a somewhat produced apex. All are rather heavily sclerotized. Above the antennae, however, there is a rather large and plainly differentiated patch of smaller, more slender and transparent type 2 scales, each armed with a long apical spine. The prestomal teeth are smaller than in *Lispa* spp.

Limnophora tetracheata Mall. and *Lasiops melanderi* Hall. Similar to *pallipes* but types 2 and 4 scales appear to be similar in structure through the ptilinum, which is bare in the fold above the lunule as in *Fannia* spp. Ptilinum very fragile.

(Coenosiinae). *Coenosia cilicauda* Mall. and *anthracina* Mall. Scales deep gray-black, type 3, apex slightly produced, a few approach type 4. The rather long-triangular frontal lunule is sharply demarked from the ptilinum which is wrinkled and bare along its margin.

(Anthomyinae). In this subfamily, only, are the scales light yellow to unpigmented and all are of types 1 to 2 with a few merging to type 4. The ptilinum is noticeably more robust than it is in other subfamilies.

Hylemyia antiqua Mg. Fig. 36. The transparent scales are typical type 1 to 2, with a few merging into type 4. To a varying degree a lateral area, which underlies about 50 scales on each side of the ptilinum, is stained from a light to a deep opaque brown. Around its edges, detached dark areas underlie individual scales but, in the center of each area, the entire membrane is uniformly stained.

Hylemyia cerealis Coq. Resembles *antiqua* but with only a light median staining above the lunule.

Pegomyia connexa Stn. Resembles *antiqua* but scales are yellow and show a greater tendency to be transformed into type 4.

MUSCIDAE

General

All species examined possessed normal type 1 to 2 scales with little tendency to be transformed into type 4. The frontal suture terminates opposite to or well above the vibrissae, but the ptilinum is usually sclerotized on the lower half of the face. The frons is armed to the vertex with rodlike structures obviously derived from fused scales. The face is unarmed, while the buccal bladders usually carry rather well-developed mammiform scales.

Musca domestica L. Fig. 37. Scales dark gray, not suggesting heavy sclerotization; types 1 and 2, with tendencies towards 4. They are densely crowded on the whole of the ptilinum except at the apex. The ptilinum is only moderately robust. Rods of "fused" scales extend on the frons to the vertex.

Muscina stabulans Fall., *Stomoxys calcitrans* L., and *Lyperosia irritans* L. are all similar to *domestica* though the scales are slightly longer and are definitely more heavily sclerotized. They are largest in *irritans* and tend to fall into diagonal rows with a higher frequency of type 4, while the bare wrinkled area immediately under the lunule, which was first observed in *Fannia* spp., is pronounced in *stabulans*.

Orthellia caesarion Mg. The type 3 scales are more slender in proportion to their length than are those of the preceding genera and, though of a gray color, they appear to be less heavily sclerotized.

GASTEROPHILIDAE

Gasterophilus intestinalis deG. Fig. 38. Scales small, type 1; evanescent basally, unpigmented. They are extremely numerous over the entire ptilinum, overlapping slightly laterally and for about one-third of their length. The ptilinum is very large and it hangs downward when at rest much as does that of the helomyzid illustrated in Fig. 11. The frontal suture terminates indefinitely at about the lower level of the eye-margins but the genae, with the intervening membranous "buccal" area, are almost completely clothed with unmodified "ptilinal" scales. It is estimated that there must be approximately 150,000 of them on the entire area.

CALLIPHORIDAE

General

In all species examined, with the exception of *Pollenia rudis* in which it reaches almost to the vibrissae, the frontal suture terminates at about halfway between the antennal fossae and the vibrissae. Scales, however, continue beyond its ends and flare outwards over much of the genae where they tend to fuse together. They extend, also, on the frons to the vertex, again with a tendency to fuse. The face is scaleless, though the buccal bladders may be moderately heavily armed with small mammiform scales. The majority of ptilinal scales are of type 2 with tendencies to vary to other normal types. There is a varying degree of scale fusion on the ptilinum itself. The latter is moderately robust, of a medium size, and is always stained brown near the extremities of the suture.

Phormia regina Mg. Fig. 39. At the time of emergence, the scales are unpigmented, but they turn to yellowish–brown after a few hours. At this time, the ptilinum is moderately strong and could be readily inflated. Among captured specimens classified as this species there was some variation. In the most extreme case, as illustrated, the blackish scales over much of the ptilinum were fused into compound rods and the ptilinum was unusually thin. Possibly the latter characteristic is correlated with the age of the individuals though similar variations have not been observed in other species.

Protophormia terra-novae R.D. Scales gray, majority type 2, merging to 1 medially. Laterally there is a slight tendency to scale fusion.

Cynomyia (*Cynomyopsis*) *cadaverina* Desv. (Fig. 40) and *Calliphora vomitoria* L. Scales type 2 to 3, with a distinct tendency to approach type 4 laterally; bases tending to flare basally; rather more refractive than are those of other species; light gray to brown. Normal.

Pollenia rudis Fab. Scales uniform type 2 to 3; dark gray; ptilinum sclerotized from about halfway between the antennal fossae and the vibrissae. Though these are parasites of earthworms the scales are not larger than are those of cadavorous species.

SARCOPHAGIDAE

General

The ptilina and armament closely resemble those of Calliphoridae. The chief difference is that, in all species examined, the suture extends to a point about opposite to the vibrissae and its contained ptilinum is membranous and unpigmented to its extremities. Despite differences in larval habits, the ptilina and their scales are very uniform. Among the latter, type 2 predominate

with a varying tendency to type 4 medially. There is a marked tendency for them to be laid down in diagonal, slightly overlapping, rows. Separate or fused scales extend on the frons to the vertex, but not on the face. The buccal bladders bear numerous, but weak, "villi".

Sarcophaga l'herminieri Desv. Fig. 41. A dung inhabiting species. The medium-brown scales are relatively smaller and more numerous than are those of species which parasitize grasshoppers.

Sarcophaga nearctica Park. Supposedly sarcophagous; scales as in *l'herminieri* but darker in color.

Sarcophaga (*Kellymyia*) kellyi Ald. and *S.* (*Protodexia*) *hunteri* Hgh. Grasshopper parasites. The ptilina are relatively more voluminous than they are in the larger species while the scales may be, in actuality, slightly longer with less tendency to be transformed to type 4.

Wohlfahrtia opaca Coq. A myiasis producing species. The light brown scales are normal type 1 to 2; possibly more approach type 4 than in *Sarcophaga*. The ptilinum is stained in the somewhat abbreviated lateral sutures.

Agria affinis Fall. Rhopaloceran parasite. The dark brown scales are less crowded than they are in other genera.

METOPIIDAE

Metopia leucocephala Rossi. Fig. 42. Bee parasite. Scales chiefly type 1, relatively larger and distinctly wider than in Sarcophagidae. The medium-brown scales are rather densely crowded on the voluminous ptilinum which, also, is stained a light brown.

CUTEREBRIDAE

Cuterebra tenebrosa Coq. Fig. 43. Despite the unusually large size of this fly, the ptilinal scales are relatively small; chiefly of type 2 merging to type 1 laterally and to type 4 medially. The upper surface of the majority of scales is stained to a dark brown but the central support is seen, in side view, to be unpigmented. They are uniform in size throughout the ptilinum but, medially, a few tend to assume in shape the modified type 4 which predominates in certain tachinids. The ptilinum is unusually large, its volume being due more to lateral development than to its length. The rather contracted frontal suture extends to the level of the antennal apices and it is completely filled with flexible ptilinum.

HYPODERMATIDAE

Hypoderma lineatum DeVil. Fig. 44. The scales are gray and the majority are type 2 but they vary considerably in shape and size on different areas of the ptilinum. They are largest medially where their bases are unusually flaring. A scattering of individuals are abnormally enlarged in the area bordering the ptilinal apex on which, as is usual, they are reduced in size. Many taper rather asymmetrically from base to apex. Laterally, they are less crowded than they are elsewhere, are smaller, and less deeply pigmented. The ptilinum does not extend medially to below the apices of the short antennae and it is less voluminous and rugged in texture than is that of *Gasterophilus*. Scales do not extend to the genae or to the membranous frons.

OESTRIDAE

Oestrus ovis L. The light-yellow, type 2 scales are slightly larger and more uniform in size than are those of *Hypoderma*. The suture does not extend to below the lower eye-margin. Despite this, the ptilinum is voluminous and rugged.

TACHINIDAE (LARVAEVORIDAE)

General

The ptilinal armature of the various species in this large family is of unusual interest because the habits of the host usually impose upon the escaping fly the environment from which it must liberate itself as it leaves its puparium. Of those which parasitize holometabolous hosts the majority do not escape from them until the latter have formed their own pupal protection. Among

lepidopterous hosts this protection may be completely lacking, as in Rhopalocera; more frequently it consists of a silken cocoon while, in the most extreme cases, it is a hard-walled earthen cell constructed about one to three inches below the soil surface by Phalaenidae.

The adults of many Tachinidae oviposit on leaves and depend either upon their liberated larvae successfully attaching themselves to, and entering a satisfactory host, or upon a suitable host swallowing their minute eggs with its food. Their first biological problem thereafter is that the larvae may be adapted to live in the large variety of hosts into which they may thus gain a somewhat fortuitous entrance. The writer (12) has discussed this problem in connection with certain species which could, or could not, be reared in different species of Phalaenidae. Later, it is essential that, should the larvae succeed in maturing in a specific host, their adults shall be equipped to penetrate its pupal protective covering.

From the following observations, it appears to be self-evident that the type of ptilinal armature possessed by different species of tachinids constitutes an important factor in determining this ability. It can hardly be maintained that the relatively enormous scales possessed by species which are known to escape from subterranean phalaenid cells were evolved as a "ready-made" adaptation with this end in view, but it would appear that only those species which are so equipped stand much chance of becoming successful parasites of hosts which form earthen cells prior to the escape of their larvae.

An examination of a number of Tachinidae indicates that the majority of species possess ptilinal scales which, though rather longer than are those in related families such as Calliphoridae, do not reveal other modifications. They are not very heavily sclerotized and are chiefly of type 3 with only a normal complement of short moderately vertical type 4 scales.

Information regarding the known hosts of many tachinids is available from a variety of sources. The most complete list is the catalogue prepared under the direction of W. R. Thompson (13), while, for local species, Essig's book *Insects of Western North America* is a useful source of additional data. It is, however, most unlikely that, even in the case of the best-known species, we possess information regarding the entire range of hosts which they parasitize successfully. Their relationships have, in almost every case, been recorded only in so far as they concern hosts which are of particular economic interest.

Species which are believed to be limited to essentially superterranean hosts, such as Orthoptera, Hemiptera, butterflies, and leaf-eating moth-caterpillars possess unmodified ptilinal scales. Even in the case of *Doryphorophaga doryphorae* Ry., which may escape from the subterranean pupal cavity of the potato beetle, the scales are normal in structure and size. Certain species which are known to parasitize subterranean phalaenid larvae, also, have normal scales. We do not know in what stage of host development the larvae of these species kill their hosts but suspect that they do so before the latter form their pupation cells.

The ptilina of all species in genera in which it is known that the adult tachinid normally escapes from a subterranean phalaenid cell, however, are armed with extremely powerful scales on the median dorsal surface (Figs. 6 and 7). They are only slightly smaller on the corresponding ventral surface. These scales are invariably very heavily sclerotized here though, laterally, they are usually much smaller and are frequently unpigmented. With the exception of species in the genus *Echinomyia* (*Fabriciella* or *Cnephaliodes*) in which they are of type 3, these are greatly lengthened and semidecumbent type 4 scales.

Field-captured representatives of many of these species reveal that the apices of a varying percentage of these enlarged scales are lacking and have every appearance of having been "eroded" away rather than having been broken off. This, it is assumed, is due to their having been vigorously employed in scraping through the earthen wall of a pupation cell.

Frontal scales, generally, are weak and appear to be nonfunctional; those on the face when present are villiform.

The few examples discussed below are arranged in accordance with the manner in which the habits of their known hosts may result in increasing difficulty for the adult tachinids to penetrate their pupal protection. It is unfortunate that generic concepts in this large family are still in a state of flux. In the following list, species are recorded under generic names which, until recently, have been most frequently employed. These are followed by alternative names (in brackets) under which they may also be recorded in the literature.

Gymnosoma (*Rhodogyne*) *fuliginosa* Desv. Fig. 45. A bug parasite. The scales are uniform, elongate type 3, with very few slightly modified to type 4; relatively small. They are light brown in color and, apparently, are only lightly sclerotized.

Acemyia (*Euacemyia*) *tibialis* Coq. A grasshopper parasite. The scales resemble those of *Gymnosoma* but are more gray than brown.

Zenilla futilis O.S. Fig. 46. A butterfly parasite. The scales resemble those of *Gymnosoma* but with a much greater tendency to be modified to type 4. They are distinctly more heavily sclerotized, moderately crowded, and overlap for about one-third of their length.

Zenilla affinis Fall. (*Aplomya estigmensis* Sell.). An arctiid parasite. The scales resemble those of *futilis* but are a little longer and more crowded, overlapping for about half their length.

Winthemia quadripustulata Fab. and *Frontina* (*Achaetoneura*) *frenchi* Will. Rather general parasites of Lasiocampidae, Arctiidae, Sphingidae, &c. Similar to *Zenilla* spp.

Hystricia (*Bombyliomyia*) *abrupta* Wied. An arctiid parasite. The scales on the ptilinum of this excessively bristly fly resemble those of *Zenilla* and they are, relatively, no larger.

Doryphorophaga doryphorae Ry. A beetle parasite, including *Leptinotarsa*. The scales are not very heavily sclerotized but are wider than are those of any preceding species and they completely conceal the ptilinal membrane over much of its surface.

Belvosia bifasciata Fab. A saturniid parasite. This is the largest tachinid taken locally. The narrow scales are heavily sclerotized and are essentially of type 3 with tendencies, over the whole to the ptilinum to approach type 4. They are relatively larger than in any of the foregoing species.

Tachinomyia variata Curran. A liparid parasite which has, also, been recorded as having parasitized the phalaenid *Agrotis orthogonia*. The ptilinum is voluminous and pendulous when withdrawn. The scales are not modified or enlarged and they resemble those of *Zenilla*. In one specimen examined a few scales had the apices missing but they appeared to be broken rather than eroded.

Aphria ocypterata Twns. A parasite of *Euxoa*. The scales of this rather small fly resemble *Zenilla*. Information is lacking as to when *T. variata* and this species kill and leave their hosts. We suspect that they do so before the latter have formed pupation cells.

Echinomyia spp., later synonymized with *Fabriciella* and *Cnephaliodes*, and still later separated into several genera. These appear to represent an intermediate stage between those

genera in which the scales are unmodified and those which possess the relatively enormous type 4 scales to be discussed later. All, in so far as is known, are phalaenid parasites. In all species of this genus which we have examined, the scales are distinctly enlarged medially but to a less extent than are those of the following genera. They are, however, typically of type 3, with slight tendencies towards type 4 near the lower edge of the ptilinum. *Echinomyia* (*Rhacogaster*) *algens* Wd. Fig. 47, a parasite of cutworms and *E.* (*R.*) *latifrons* Toth., reared from *Agrotis orthogonia* Morr., exemplify this genus. Several specimens examined showed abrasion of the scale apices.

Epalpus (*Argenteopalpus*) *signiferus* Wlk. Fig. 48 is a locally abundant early-spring flyer which is recorded as parasitizing a phalaenid, *Lithophane* sp. This is an example of a tachinid which possesses flattened type 4 scales on the upper and lower median surfaces of its ptilinum. They are, however, smaller in this species than in any other with this character which we have examined.

Peleteria (*Peletieria*) spp. Host records of species in this genus indicate that they are essentially phalaenid parasites. It has recently been divided into a number of separate genera. *Peleteria* (*Sphyromyia*) *campestris* Curr., a parasite of *A. orthogonia*, exemplifies the generic ptilinum and it resembles that of *Gonia* S.L., discussed below.

Bonnetia (*Linnaemyia*) *compta* Fall. An abundant parasite of *A. orthogonia* Morr. and other Phalaenidae. It normally kills its host after the later has formed its pupation cell and has entered the prepupal stage. Its scales resemble those of *Gonia* spp. They are among the largest we have observed. Among captured specimens, a number of these large scales have the apices missing.

Gonia spp. This large group of undoubtedly closely related flies has recently been separated into several genera. They are essentially phalaenid parasites which allow their hosts to pupate before killing them. All of the species with which we are familiar are univoltine and hibernate in their hosts' pupal cells. It is many years since the writer had an opportunity to study the habits of these flies in the field but, in so far as he can remember, adult moths always escaped from their cells by a reasonably circular hole whereas tachinids did so through a jagged opening.

This genus, however, contains one species *Gonia* (*Knabia*) *frontosa* Say. which, we believe, is normally a parasite of tree-foliage eating caterpillars and it pupates in the aerial cocoons of its hosts. In common with related species, its ptilinum is armed with greatly enlarged type 4 scales. Since these are as large as are those of many which parasitize Phalaenidae it would appear that, should its larva be capable of developing in cutworms, the adult should have no difficulty in penetrating their pupation cells.

Gonia (*Knabia*) *frontosa* Say. Parasite of *Malacosoma disstria* Hub. Enlarged, heavily sclerotized, decumbent type 4 scales are confined to the upper and lower median surfaces of the ptilinum. Around its periphery, as well as at its apex, they gradually reduce in size and pigmentation. Apically, they are very short vertical type 4, about one twentieth the length of the enlarged dorsal scales; around the periphery the very light-yellow scales are of type 3 about one tenth the length of the dorsal ones. As is the case with all tachinids with enlarged scales, those on the dorsal half of the ptilinum are about 10% longer than are those on the lower half. There was no abrasion of the scale apices on any specimen that was examined.

Gonia (*Reamuria*) *aldrichi* Toth. A parasite of *Agrotis orthogonia* Resembles *G. frontosa*, scales about one and one-half times as long. It is believed that the inflated ptilinum shown in Fig. 6 represents this species.

Gonia (*Reamuria*) *longiforceps* Toth. Fig. 49. A parasite of *A. orthogonia*. No species examined possessed larger scales. These are somewhat variable in size and the largest one observed is illustrated. Its apex is missing. In this species the scales at the ptilinal apex are less inclined to tend towards type 4 than they are in *frontosa*. A ptilinal seta is included in the drawing to show its comparative length. On account of lack of pigmentation in all lateral scales, including those in the lateral sutural grooves, a gross examination of the ptilinum gives the impression of its being transparent with a wide median black band which does not quite reach its upper and lower margins.

HIPPOBOSCIDAE

General

In two species examined the frontal suture is wide and transverse, as it is in many Tetanoceridae. It bends down slightly at its extremities. The ptilinum is ribbonlike but tends to be sclerotized. It folds inwards by a single fold along its equatorial area and does not appear to be capable of protrusion.

Melophagus ovinus L. Fig. 50. Medially the ventral scales are asymmetrical and somewhat bractlike. Laterally they become increasingly narrowed and, finally, fuse to form longitudinal flattened "rods". All dorsal scales are fused to form horizontal, irregularly wrinkled and flattened rods. The entire ptilinum is sclerotized.

Ornithomyia fringillina Curt. Resembles *M. ovinus* but the scales are less definitely outlined and appear to fuse together to form "zig-zag" transverse bands which cover the greater part of the more flexible ptilinum. Rarely, only, can the outline of a sharply tapering asymmetrical scale be distinguished.

The Relative Size of Ptilinal Scales in Different Species of Flies

Extreme specific variations in the size of flies obviously affects the actual length of their ptilinal scales. This can be inferred from the accompanying illustrations, all of which have been drawn with a camera lucida at a magnification of 500. More significant, regarding possible function, is their size in relation with that of their possessors. In gauging this, the best method appears to be to record their length in proportion to the width of the flies' heads. All measurements are given in millimeters. In order to save space, data are grouped where variations in proportion appear to have little significance. The species included in each group can, however, be deduced from the foregoing individual discussions of them.

No significant sexual difference was observed either in head-width or scale-length. Scales, however, vary in size on different parts of the same ptilinum. Attempts were made to measure the average length of those near the median center of the upper half of the ptilina. It was not always possible to pin point this area in our dissections while extensive overlapping of scales here frequently rendered exact measurements impossible. Furthermore, in many species, the bases of the scales are evanescent. Measurements, as given, must therefore be considered as approximate. Errors much in excess of 10% are improbable, but this possibility exists.

For scales which terminate in an elongate apical bristle, the lengths, as given, refer to that of the body only. Because of their unusual structure, scale lengths in the ephydrid, *O. mantis*, several Tetanoceridae, and all species in the anthomyid genus, *Fannia*, are hardly comparable with those of more typical flies.

Despite these inherent shortcomings, the list indicates for each species the extent to which any unusual variation in size might, in some manner, be correlated with its ability to escape, as a recently emerged adult, from its immediate surroundings.

Family	Species	Scale length	Head width	Ratio, scale: head
CONOPIDAE	4 species	0.014–0.021	2.08–2.90	0.005–0.009
	Z. fulvifrons	0.021	1.70	0.012
OTITIDAE	3 species	0.014–0.015	1.50–2.14	0.007–0.010
TRUPANEIDAE	3 species	0.017–0.019	1.42–1.95	0.010–0.013
PALLOPTERIDAE	*P. jucunda*	0.014	1.00	0.014
LONCHAEIDAE	*L. polita*	0.019	1.66	0.011
TANYPEZIDAE	*T. luteipennis*	0.018	1.39	0.013
CALOBATIDAE	*C. nasoni*	0.013	0.92	0.014
MICROPEZIDAE	*M. lineata*	0.013	0.81	0.016
PIOPHILIDAE	*P. affinis*	0.010	1.03	0.010
SEPSIDAE	2 species	0.014–0.017	0.94–1.03	0.015–0.017

Family	Species	Scale length	Head width	Ratio, scale: head
LAUXANIIDAE	2 species	0.012–0.016	1.09–1.25	0.010–0.015
DROSOPHILIDAE	3 species	0.005–0.010	0.67–1.00	0.007–0.010
ASTEIIDAE	*A. multipunctata*	0.008	0.69	0.012
OPOMYZIDAE	*A. tenuis*	0.008	0.61	0.013
AGROMYZIDAE	5 species	0.008–0.013	0.47–0.86	0.012–0.019
	A. angulata	0.019	0.72	0.026
PHYLLOMYZIDAE	4 species	0.004–0.006	0.64–1.03	0.006–0.008
CHLOROPIDAE	3 species	0.006–0.007	0.61–1.00	0.006–0.010
EPHYDRIDAE	2 species	0.014	1.39–1.53	0.009–0.010
	O. mantis	0.004	1.69	0.002
	P. nitida	None	1.50	—
BORBORIDAE	*B. equinus*	0.008	1.03	0.008
CLUSIIDAE	*C. melanostoma*	0.010	0.89	0.011
CHAMAEMYIDAE	*C. polystigma*	0.014	0.78	0.005
TETANOCERIDAE	2 species	0.010–0.011	0.92–1.16	0.009–0.011
	3 species	Not comparable with others	1.39–1.66	Not comparable with others
	Sepedon spp. (5)	None	1.26–1.72	—
CHRYOMYIDAE	*T. fumipennis*	0.008	0.72	0.011
PSILIDAE	*P. perpolita*	0.009	0.83	0.011
COELOPIDAE	*C. frigida*	0.004	0.97	0.004
HELOMYZIDAE	5 species	0.009–0.014	1.03–1.42	0.007–0.012
DRYOMYZIDAE	*N. anilis*	0.012	1.44	0.008
CORDYLURIDAE	*C. confusa*	0.015	1.66	0.009
	A. varipes	0.021	1.25	0.017
SCOPEUMATIDAE	2 species	0.017–0.018	1.61–2.19	0.008–0.011
ANTHOMYIDAE	Phaoninae (2)	0.017–0.022	1.84–2.17	0.009–0.010
	Mydaeinae (2)	0.022–0.023	2.09–2.25	0.010–0.011
	Hydrotaeinae (1)	0.018	2.22	0.008
	Faniinae *A. gibbera*	0.009	0.72	0.013
	6 species of *Fannia*	0.002–0.004	1.09–1.84	0.002–0.003
	Lispinae (2)	0.017	1.88–1.94	0.009
	Limnophorinae (3)	0.013–0.017	1.42–1.64	0.009
	Coenosiinae (2)	0.014	0.83–1.16	0.012–0.017
	Anthomyinae (4)	0.012–0.014	1.31–1.75	0.008–0.011
MUSCIDAE	4 species	0.014–0.017	2.28–2.67	0.006–0.007
	L. irritans	0.014	1.16	0.012
GASTEROPHILIDAE	*G. intestinalis*	0.019	3.94	0.005
CALLIPHORIDAE	6 species	0.014–0.016	2.75–3.84	0.004–0.005
	P. rudis	0.018	2.91	0.006
SARCOPHAGIDAE	4 species	0.016–0.022	2.30–3.70	0.005–0.007
	S. kellyi and *hunteri*	0.017–0.019	2.03–2.47	0.008
METOPIIDAE	*M. leucocephala*	0.020	1.50	0.013
CUTEREBRIDAE	*C. tenebrosa*	0.029	9.50	0.003
HYPODERMATIDAE	*H. lineata*	0.021	4.10	0.005
OESTRIDAE	*O. ovis*	0.025	3.75	0.007
TACHINIDAE	12 species. Hosts superterranean	0.071–0.096	1.84–6.00	0.006–0.016
	T. variata	0.031	3.00	0.010
	A. ocypterata	0.029	3.00	0.010
	E. algens	0.076	4.00	0.019
	E. signiferus	0.116	3.50	0.033
	G. frontosa	0.130	3.20	0.041
	3 species of *Gonia*	0.130–0.215	3.20–4.00	0.041–0.054
	Phalaenid pupae			
	P. campestris	0.177	3.80	0.047
	B. compta	0.190	3.50	0.054
DEXIIDAE	*P. tibialis*	0.021	2.35	0.009
HIPPOBOSCIDAE	*M. ovinus*	0.014	1.25	0.011
	O. fringilaria	0.010	1.25	0.009

In the foregoing list it will be seen that, on the ptilina of flies, representing the majority of families, the ratio of head width to scale length varies between 1 : 0.005 and 1 : 0.015, which is approximately ×200 to ×66. Only in species in which the scale structure is abnormal is this ratio appreciably exceeded. On the other hand, apart from in certain Tachinidae, it appears to be significantly reduced only in certain Agromyzidae, e.g. *A. angulata* (1 : 0.026 or ×40) and, possibly, though to a lesser degree, in Anthomyidae, subfamily Coenosiinae.

Among those species of Tachinidae which penetrate phalaenid pupation cells, however, it may be reduced to as little as 1 : 0.054, or approximately ×20. This is approached in at least four genera which are not particularly closely related to each other.

Discussion and Summary

From an examination of about 150 species of Schizophora, it is evident that the ptilina of this entire group normally are armed with thousands of minute, to occasionally very large, cuticular projections to which the name "scale" has been applied. Only in 2 of the 40 families examined were any species found in which they are lacking.

In size, the vast majority vary in length from about 1/200 to 1/66 of the width of the head, i.e. 0.005 : 1 to 0.015 : 1.

The apices of these scales are directed upwards and backwards on the dorsal half of the ptilinum and downwards and backwards on its ventral half. On a narrow transverse band, which is about six scale-lengths in width and which passes through the ptilinal apex, they are vertical and are usually reduced in length. Underlying this band retractile muscles are attached to the inner wall of the ptilinum.

Scales vary in structure in different families but, in general, all appear to be derived from a "primitive" type which, in side view, resembles a rose-thorn. From above, the dorsal surface is seen as a widened blade. Variations in its outline can be likened to a wheat, barley, or oat grain respectively. The most frequently encountered variant from this general type is conical and is strengthened from its base to its apex with about six heavily sclerotized rods. These give it the appearance of a partially collapsed "bell" tent. Scales which are intermediate in structure between these two types are not uncommon on all ptilina on which the latter are represented.

On the ptilina of a few widely separated families these normal types are entirely replaced with others which, when first observed, appear to have no relationship with them. Cuticular scales are of wide occurrence on the connective membranes of Arthropoda and they assume a great variety of forms. Elsewhere than on ptilina they usually are small and rather widely scattered. Particularly among the Acalyptrata, however, the buccal bladders at the base of the proboscis may be as heavily armed with scales resembling those of the ptilina as are the latter, themselves. On the proboscis of the

conopid, *P. furcillata* Will., these crowded scales undergo a gradual transformation, between its base and its apex, which includes nearly all of the infrequently encountered variations which are peculiar to the ptilina of different families. This indicates that all are variations of the same structure and may throw some light upon their possible functional adaptations.

In the majority of ptilina both the structure and the disposition of scales indicate that they may be capable of being employed, after the fly has escaped from its puparium, in assisting it to penetrate obstructions, such as moderately consolidated soil. The actions of several species of Calliphoridae in attempting to penetrate glass at this time support this view, despite the fact that, in these flies, the scales are only about 0.005 of the head-width and they belong in the group in which they are of the minimum normal size.

As soon as the ptilinum encounters glass, while the fly is leaving its puparium, it explores the surface of the obstruction. Following this, the apex is withdrawn by muscular contraction to produce a temporary transverse fold which brings together along its edges the centers of the upper and lower surfaces of the ptilinum. Both surfaces (together with the frons) are pressed firmly against the glass. Greatly increased internal blood pressure follows and is accompanied by a gradual release of the ptilinal apex. This causes the tips of the opposed scales on the two surfaces to be forced apart and to scarify the obstruction. This scarifying action may be repeated dozens of times during the next few hours. It is obviously "purposeful". After about an hour of fruitless endeavor, about 50% of the flies under observation released a copious fluid from their pseudotracheae. This flooded the obstruction and probably would soften certain types. Its liberation was invariably followed by extreme exertion. Its use appears, however, to entail some risk, unless, as a result of its employment, a rapid escape is effected. On evaporation, it firmly cements small particles together.

In many species of flies scales extend from the ptilinum to other parts of the epicranium. This applies, chiefly, to the frons in Calyptratae or to the face in Acalyptratae. Less frequently, they flare out from the lower ends of the frontal suture and cover the genae. Epicranial scales tend to be rather smaller than are those on the ptilinum and they are frequently fused together longitudinally. Prior to its sclerotization, those on the frons are definitely employed by Calliphoridae in conjunction with the ptilinum in efforts to escape. In Agromyzidae the frons may be exceptionally heavily armed with separate scales. It retains its flexibility throughout the life of the fly and is, undoubtedly, functionally a part of the ptilinum.

Powerful armature on the buccal bladders is more common among Acalyptratae than it is in Calyptratae. The latter rarely possess more than a scattering of "villi", or similar weak structures, on this area. This applies to Calliphoridae. Despite this, these flies inflate them to the utmost whenever they make a major effort to penetrate an obstruction with their ptilina. Though they may occasionally be brought against the glass and forced over its surface they do not appear to be put to any specific use. It would appear

that Acalyptratae in which these structures are heavily armed with scales may have a definite use for them. No flies, so equipped, have, however, been observed attempting to penetrate an obstruction.

In a few, unrelated, species the ptilinal scales are abnormally large in size and this character is usually accompanied by their being unusually heavily sclerotized. This combination is most pronounced in those species of Tachinidae which normally allow their phalaenid hosts to construct subterranean pupation cells before their larvae kill them. These flies must, therefore, ultimately penetrate these cells by their own efforts. That they do so with the aid of their ptilinal scales is indicated by the relatively enormous development of these structures on all species which are known to have this habit. In several of these the length is over 0.05 of the head width. This is, at least, 10 times their relative size in Calliphoridae. Not only does their size and structure suggest that they are particularly well-adapted to powerful digging but the fact that many field-captured adults have the tips of many of them abraded suggests that they have, undoubtedly, been employed for this purpose.

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** Papers marked with an asterisk have not been seen by the writer.*

Explanation of Figs. 12–50

All illustrations of individual scales have been made, with the aid of a camera lucida, at a magnification of 500. The text explains whether each illustration refers to the prevailing type of scale for the species concerned or whether it is of some unusual modification which is confined to part of the ptilinum. Scales drawn with their apices directed upwards are from the upper half of the ptilinum and those with it downward are from its lower half.

Unless otherwise stated, average-sized scales were selected for drawing. Where more than one scale is illustrated from the same ptilinum the first description refers to the one on the left.

CONOPIDAE: FIG. 12. *P. furcillata* Will. Lower and upper outlines of a ventral scale as seen from above. FIG. 13. *M. vicaria* Walk. Upper and side view of scale.

OTITIDAE: FIG. 14. *S. vibrans* L. Upper and side view of scale. FIG. 15. *E. myopaeformia* V.R. Upper and side view of scale.

TRUPANEIDAE: FIG. 16. *E. solidaginis* Fit. Imbricated scales near ptilinal apex, and a normal type 3 on its upper surface.

LONCHAEIDAE: FIG. 17. *L. polita* Say. Type 4 scale from median ventral ptilinal surface and "rods" of fused scales from near the genal angles.

TANYPEZIDAE: FIG. 18. *T. luteipennis* K. & S. Normal scale and one with flared base from lower half of ptilinum.

SEPSIDAE: FIG. 19. *T. putris* L. Bractlike scale from above frontal lunule and fused scales near upper edge of ptilinum.

DROSOPHILIDAE: FIG. 20. *D. melanogaster* Mg. Indefinite scales near center of ptilinum. Laterally, and near genal angles they are better defined, as shown.

AGROMYZIDAE: FIG. 21. *A dubitata* Mall. Scale with divided apex from apex of ptilinum and an asymmetrical one from near this area. FIG. 22. *A. angulata* Lw. Upper and side view of one of the larger scales.

CHLOROPIDAE: FIG. 23. *O. frit* L. Upper and side view of scale. This was the smallest species of fly dissected.

EPHYDRIDAE: FIG. 24. *P. turbida* Curr. Upper and side view of typical scale and upper view of one from just above the frontal lunule. FIG. 25. *O. mantis* DeG. Portion of ptilinum from near its center showing comb-scales on dorsal one-third and intensive wrinkling of ventral two-thirds.

CHAMAEMYIDAE: FIG. 26. *C. polystigma* Mg. Variations in ptilinal scales, all of which are of unusual shapes.

TETANOCERIDAE: FIG. 27. *M. schoenheri* Fall. Normal scales on a normally developed ptilinum. FIG. 28. *T. valida* Lw. "Jigsaw puzzle" type of scales which cover the greater part of a ribbonlike ptilinum and a side view of those near its upper margin. FIG. 29. *L. saratogensis* Fit. Imbricate scales near upper center of ribbonlike ptilinum, and one on lower half.

COELOPIDAE: FIG. 30. *C. frigida* Fab. Circular scales which arm the entire ptilinum.

HELOMYZIDAE: FIG. 31. *S. nemorum* Mg. Partly fused scales and one of the greatly enlarged ptilinal setae near the dorsal margin of the ptilinum.

DRYOMYZIDAE: FIG. 32. *N. anilis* Fall. Upper and side view of the unusual type 4 scale which arms the entire ptilinum.

ANTHOMYIDAE: FIG. 33. *F. scalaris* Fab. This type of scale predominates on the ptilina of all species examined in the genus *Fannia* and was never observed elsewhere. FIG. 34. *L. nasoni* Stein. Upper and side view of scales which approach, in structure, the greatly enlarged scales of certain genera of Tachinidae. FIG. 35. *L. a. pallipalpis* Zett. Side view of typical scale; side and upper view of scales from a small differentiated patch just above the frontal lunule. FIG. 36. *H. antiqua* Mg. Portion of the edge of one of the lateral areas on the ptilinum which is stained black.

MUSCIDAE: FIG. 37. *M. domestica* L. Upper and side view of scales; fused scales which arm the frons.

GASTEROPHILIDAE: FIG. 38. *G. intestinalis* DeG. Upper and side view of small scales which arm the ptilinum and the buccal area.

CALLIPHORIDAE: FIG. 39. *P. regina* Mg. Upper and side view of fused ptilinal scales. This fusion and heavy pigmentation is atypical for the majority of specimens examined. FIG. 40. *C. cadaverina* Desv. Scales which are either somewhat flaring or parallel sided basally; a lateral type 4 scale.

SARCOPHAGIDAE: FIG. 41. *S. l'herminieri* Desv. Upper and side view of scale from median area of ptilinum.

METOPIIDAE: FIG. 42. *M. leucocephala* Rossi. Upper and side view of scale.

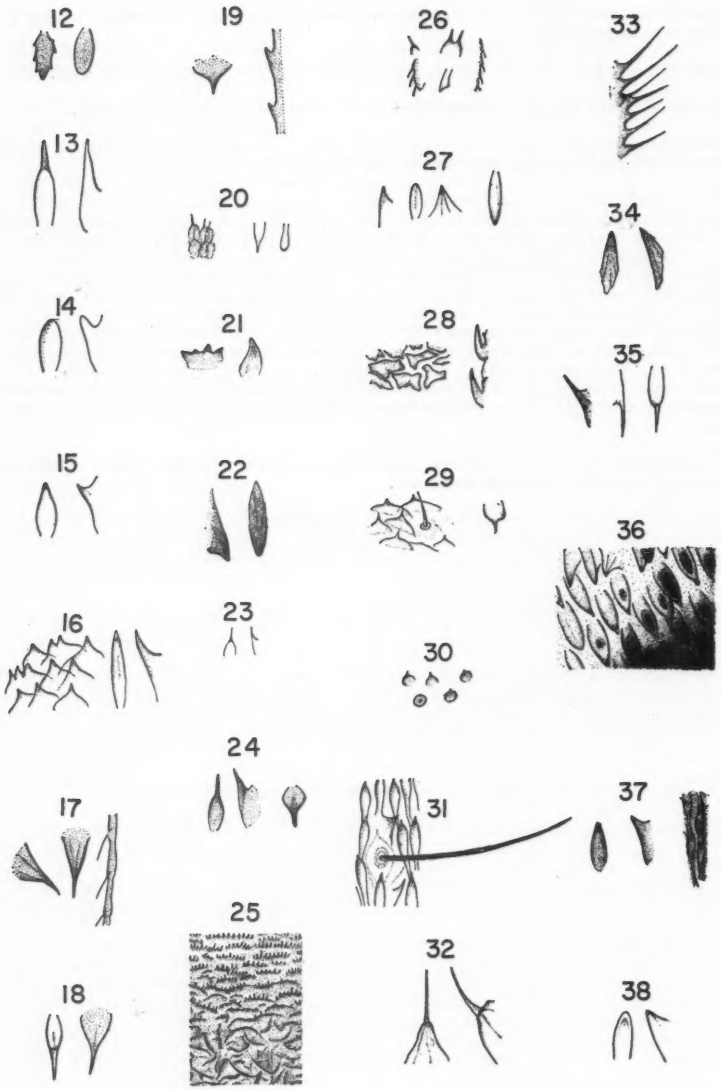
CUTEREBRIDAE: FIG. 43. *C. tenebrosa* Coq. Upper and side view of scale.

HYPODERMATIDAE: FIG. 44. *H. lineatum* DeV. Normal lateral scale; a slightly asymmetrical median scale; an individually enlarged median scale.

TACHINIDAE: FIG. 45. *G. fuliginosa* Desv. A bug parasite. Typical type 3 scales which clothe the entire ptilinum. FIG. 46. *Z. futilis* O.S. A rhopaloceran parasite. Typical type 4 scales which cover most of the ptilinum. FIG. 47. *E. algens* Wd. A phalaenid parasite. Enlarged type 3 scales which cover the upper and lower median ptilinal areas. FIG. 48. *E. signiferus* Wlk. A phalaenid parasite. Enlarged type 4 scales which cover the upper and lower median ptilinal areas. FIG. 49. *G. longiforceps* Toth. A phalaenid parasite. *a.* The largest individual type 4 scale observed in any fly though those of *B. compta* are very similar in size and structure. The apex of this scale appeared to have been abraded away while the fly penetrated the pupation cell of its host. *b.* An unpigmented lateral type 3 scale. *c.* and *d.* Two apical scales. In this species they are not modified to type 4. *e.* An unpigmented type 4 scale from near the upper ptilinal margin. *f.* A ptilinal seta drawn to the same magnification as are the scales.

HIPPOBOSCIDAE: FIG. 50. *M. ovinus* L. Portion of the ribbonlike ptilinum from near one lateral extremity and near its center.

(Note: Figs. 12-50 follow.)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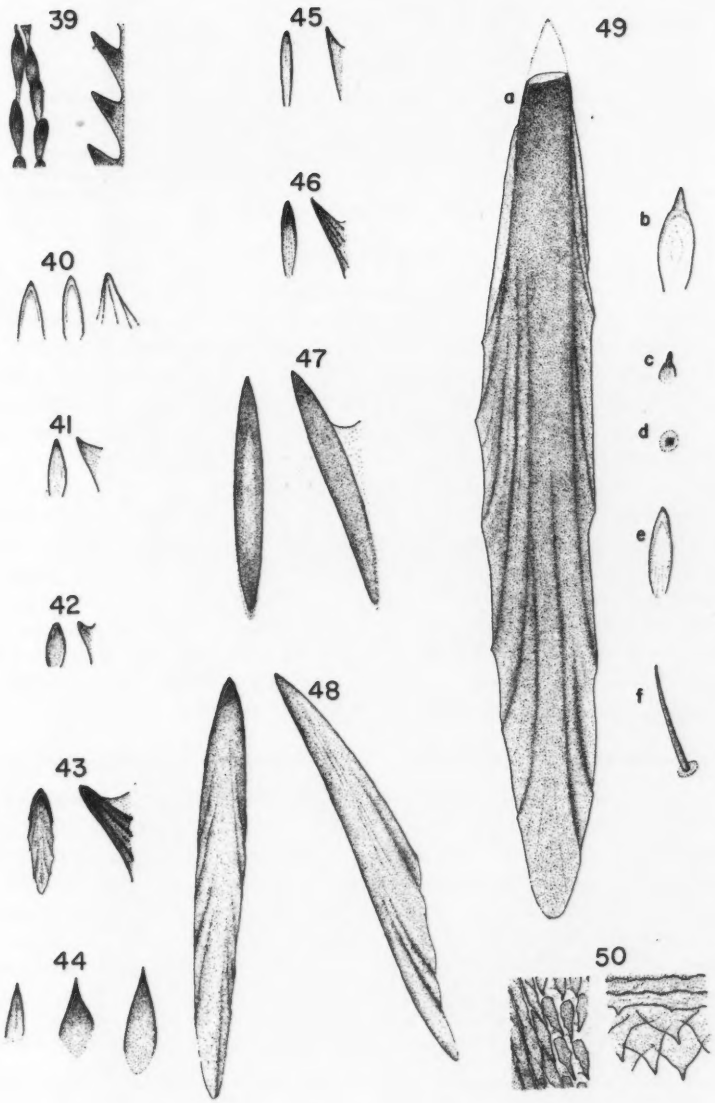
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THE EFFECT OF METAMORPHOSIS ON THE MULTIPLICATION OF AN INSECT VIRUS[1]

By F. T. Bird

Abstract

The prepupal stage of the European spruce sawfly is immune to infection by a polyhedral virus disease. The virus multiplies only in the nuclei of the digestive cells of the mid-gut epithelium and during metamorphosis these are replaced by embryonic cells not susceptible to infection although they develop into digestive cells eventually. The prepupal mid-gut, a temporary gut, is composed entirely of embryonic cells. New digestive cells appear later in the pupal and adult stages and these rapidly become infected with virus.

Introduction

Polyhedral virus diseases affect chiefly the larval stage of insect development. At some later stage of development the insect becomes immune or at least resistant to infection. Preliminary studies on the virus disease of the European spruce sawfly, *Diprion hercyniae* (Htg.) indicated that the insect becomes immune to infection soon after the cocoon is spun (1). The silkworm, *Bombyx mori* L., becomes resistant to infection in the adult stage (2) presumably because of a difference in the rate of growth of adult cells as compared with the cells at other stages of development.

This paper presents the results of studies on the histological and cytological changes associated with molting and pupation in the European spruce sawfly (Hymenoptera), and their effect on the multiplication of the virus disease of this insect. Unlike the polyhedral virus of the silkworm (Lepidoptera), the virus affecting the European spruce sawfly multiplies only in the nuclei of mid-gut cells (4). Mid-guts of normal and of virus-infected spruce sawflies were studied during molting and pupation. Tissues were fixed in Bouin's fluid and stained with Heidenhain's iron haematoxylin.

Regeneration of the Mid-gut Epithelium of Normal Insects

Two types of cells make up the mid-gut epithelium of European spruce sawfly larvae: large columnar digestive cells bounded externally by a basement membrane and internally by a striated border, and groups or nidi of small embryonic replacement cells located at various places along the mid-gut just beneath the basement membrane. As in other insects (7), new digestive cells are propagated by the regenerative nidi. In some insects (5) there is a complete replacement of the digestive epithelium at each molt, whereas in

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Contribution from Laboratory of Insect Pathology, Sault Ste. Marie, Ontario. Contribution No. 70, Division of Forest Biology, Science Service, Department of Agriculture, Ottawa, Canada.

Sections of mid-gut epithelia of fourth-instar larvae during the molting period.
FIG. 1. Shows (*a*) basophilic globules in the anterior part of the mid-gut. 450×. FIG. 2. Shows (*a*) fat vacuoles and (*b*) detached portion of the striated border in the posterior part of the mid-gut. 450×. FIG. 3. Shows nuclei containing large spindle-shaped nucleoli frequently found during the molting period. 1500×.

PLATE I

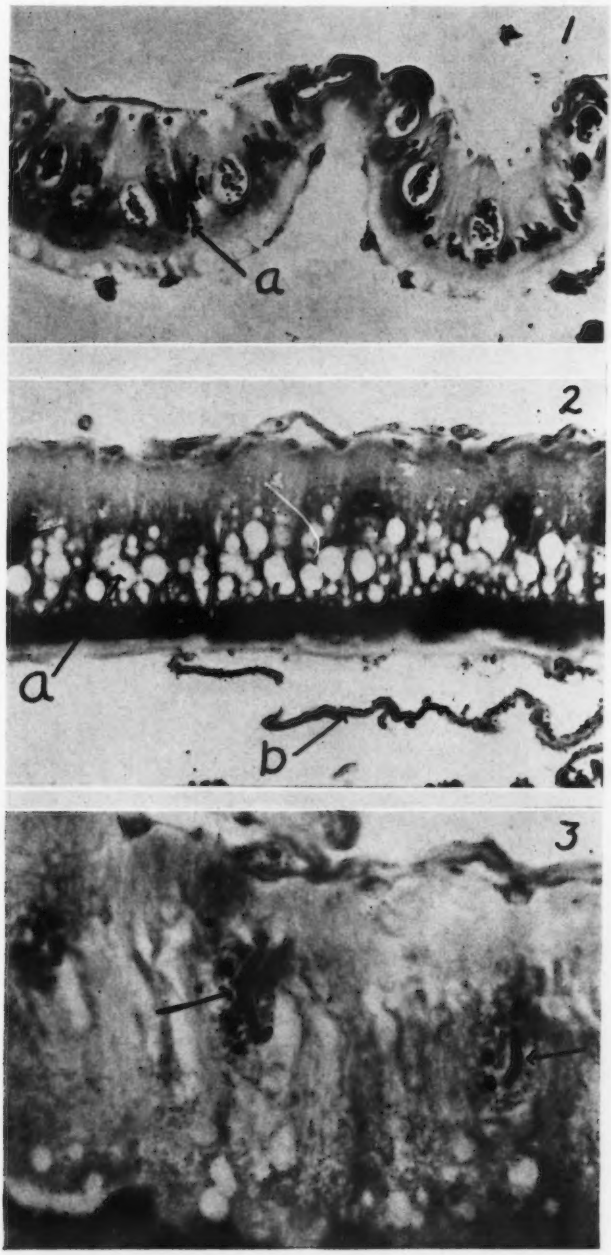
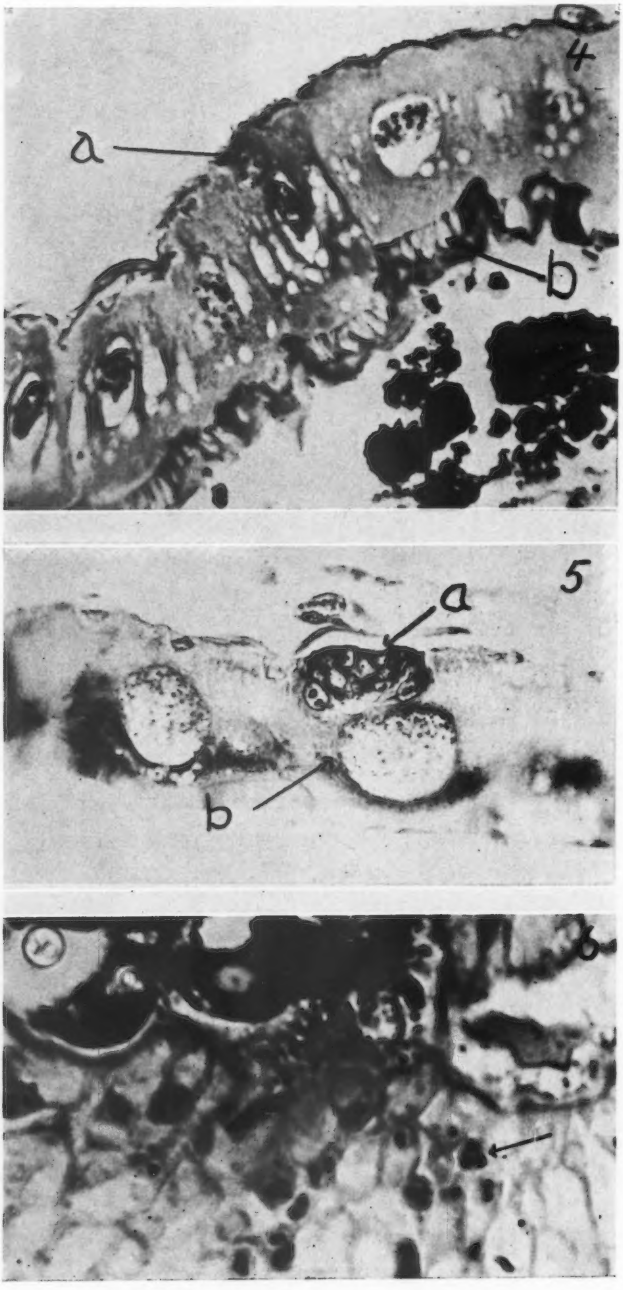


PLATE II



others (6) complete replacement takes place only during pupation although old cells may be rejuvenated and new cells added. Replacement in the European spruce sawfly is of the latter type.

At each of the first four molts of European spruce sawfly larvae, changes occur in the mid-gut epithelium which suggest that waste products are eliminated, cells are rejuvenated, and new cells are added. Prior to molting, basophilic globules accumulate in the cytoplasm of digestive cells in the anterior part of the mid-gut (Fig. 1) and fat vacuoles in the posterior part of the mid-gut (Fig. 2). These disappear during the molting period and are not found in recently-molted larvae. The striated border of the digestive cells becomes much vacuolated (Fig. 4) and parts become detached (Fig. 2), suggesting that this structure is reconstituted during the molting period. Cells containing large variously-shaped nucleoli, not found in feeding larvae, are numerous in the mid-gut epithelium of larvae during the molting period (Fig. 3). Frequently the position of these cells, in relation to the regenerative nidi, suggest that they have been propagated by the nidi although no activity of the nidi has been observed (Fig. 4). The important observation is that the digestive epithelium remains intact during the molting periods of young larvae.

At the time of the fifth larval molt, the digestive epithelium completely disintegrates and a new mid-gut is formed from cells propagated by the regenerative nidi. This process is evident from a granulation of the apical borders of the digestive cells, a later disintegration of the nuclei of the digestive cells, and an increase in the size of each regenerative nidus and in the number of cells therein (Fig. 5). After the fifth molt this process is continued in the mid-gut of the nonfeeding sixth-instar larva which, after evacuating a "yellow body", spins a cocoon. Formation of the new gut is completed by the time the insect enters the prepupal stage soon after the cocoon is spun.

The mid-gut of the prepupa is composed entirely of embryonic cells (Fig. 6). The old digestive cells have completely disappeared and new digestive cells are not formed until a later stage of development. In the event of diapause further development is arrested. Following diapause, or in the case of development without diapause, there is a further multiplication and rearrangement of cells which result in a much shorter mid-gut. Digestive cells appear in the mid-gut of the pupa and when metamorphosis is complete and the adult mid-gut is formed, the only embryonic cells are those located in regenerative nidi at the bases of the digestive cells, as in the larval mid-gut.

Regeneration of the Mid-gut Epithelium of Virus-infected Insects

Polyhedral bodies are formed within the nuclei of the digestive cells of the mid-gut epithelium of the spruce sawfly larva (4). Infected cells are found, 48 hr. after the virus is fed to the larvae, at two places in the mid-gut, one in

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Sections of mid-gut epithelia during the molting period.

FIG. 4. Shows (*a*) regenerative nidus and (*b*) vacuolization of the striated border of a fourth-instar larva. 1000×. FIG. 5. Shows (*a*) enlarged regenerative nidus and (*b*) disintegrating digestive epithelium of a fifth-instar larva. 1000×. FIG. 6. Mid-gut epithelium of a prepupa showing the embryonic type of cell nucleus. 600×.

the anterior section and one in the posterior section (Fig. 9). The disease spreads from these initial infection centers until, by 96 hr., all the nuclei of the digestive cells are filled with a dark-stained, granular mass of polyhedra. Fig. 7 is a section of the mid-gut of a larva showing cells in various stages of infection. Fig. 8 is a section of the posterior part of the mid-gut and Malpighian tubule showing cells in an advanced stage of infection.

Embryonic cells located in the regenerative nidi of the mid-gut epithelium are not susceptible to infection. Polyhedra are never formed within their nuclei although, as a result of infection of the digestive cells, they are stimulated to produce abnormal cell proliferations (3) (Fig. 10).

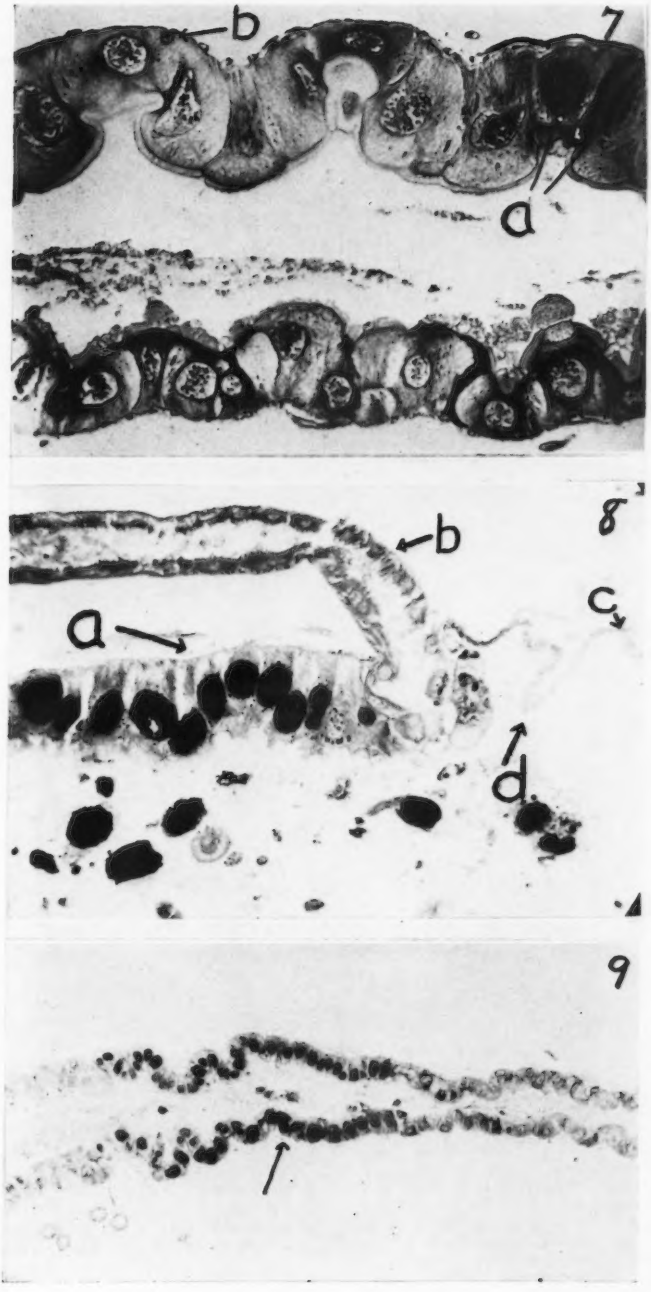
The molting of young larvae has no effect on the multiplication of the virus. Infection is delayed if an attempt is made to feed virus to the larvae at the time of molting, since larvae stop feeding during the molting period. If, however, infection of the mid-gut cells has taken place, the infection process is continued from one instar to the next. Larvae infected in the third instar, which lasts only three or four days, die from disease after molting to the fourth instar. Conversely, the infection of the mid-gut has no effect on the time at which molting takes place. Larvae molt irrespective of the degree of infection, in some cases a few hours before death.

If a larva is infected in the fifth instar and molting takes place, the infection process is continued in the mid-gut of the sixth-instar larva and after the sixth-instar larva spins a cocoon. During this time the old digestive cells are disintegrating and a new mid-gut is being formed by cells propagated by regenerative nidi. Abnormal cell proliferations are found in infected areas. Masses of proliferating cells project into the body cavity and these do not become infected with virus. Other cells migrate into the digestive epithelium and apparently develop into digestive cells which rapidly become infected with virus (Fig. 11). As a result there is a great thickening of the mid-gut epithelium in infected areas (a general thickening in the case of total infection) due mostly to the rapid propagation of new digestive cells, their destruction by virus, and subsequent accumulation. The virus infection process and abnormal cell proliferations stop abruptly when the prepupal mid-gut is formed.

The prepupal mid-gut, which is composed entirely of embryonic cells, is not susceptible to virus infection and if the disease is not too advanced the insect pupates and develops into the adult. Large masses of disintegrated cells, which accumulated in infected areas, remain within the mid-gut lumen until the development of the pupal mid-gut when, because of the development of a much smaller gut, some are pushed out through the developing gut wall and come to lie in the body cavity.

Sections of the mid-guts of larvae infected with virus.

FIG. 7. A mid-gut in an early stage of infection showing (*a*) two polyhedra-filled nuclei and (*b*) regenerative nidus. 150×. FIG. 8. Section of parts of the mid-gut, Malpighian tubule, and hind-gut showing advanced infection of the mid-gut cells: (*a*) mid-gut; (*b*) Malpighian tubule; (*c*) hind-gut; (*d*) pyloric valve. 150×. FIG. 9. Section of the mid-gut showing advanced infection at one of the initial infection centers. 30×.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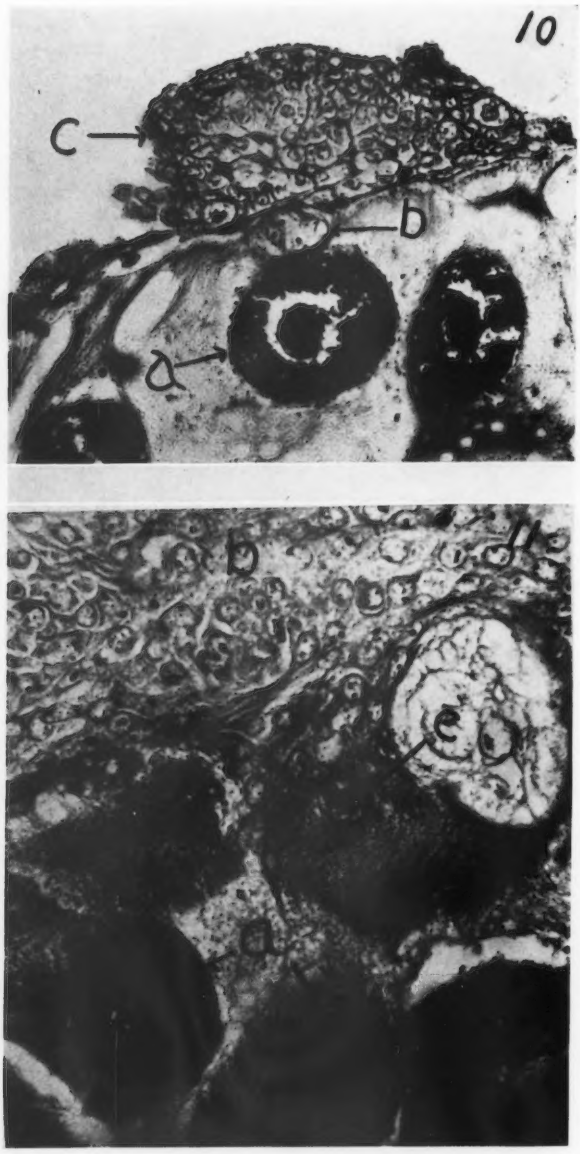

Abnormal proliferations in the mid-guts of virus-infected larvae.

FIG. 10. Mid-gut of fifth-instar larva showing (*a*) infected nucleus of a digestive cell; (*b*) uninfected regenerative nidus; and (*c*) abnormal proliferations. 600×. FIG. 11. Mid-gut of a sixth-instar larva showing (*a*) virus-infected nuclei; (*b*) abnormal cell proliferation projecting into body cavity; and (*c*) proliferating cells infiltrating the cytoplasm of digestive cells. 600×.

Infection occurs in the newly formed digestive cells of the pupa and in all the digestive cells of the adult as in the young larva. It is therefore concluded that for some reason not understood the virus multiplies only in digestive cells and not in embryonic cells, although the latter eventually develop into digestive cells. The infection process stops at the prepupal stage because the mid-gut is then composed entirely of embryonic cells but infection again occurs when new digestive cells are formed in the pupal and adult stages.

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LONGEVITY OF BLACK FLIES IN CAPTIVITY[1]

By Douglas M. Davies[2]

Abstract

Adult female black flies were maintained in captivity for as long as 63 days, 34 living for at least 20 days. The flies were kept in longevity tubes in an unheated room during the spring and summer, and survived longest when sugar was provided as granules and water by a wick. Flies of undetermined age, netted away from the stream, outlived those that emerged one day before the experiment. Females lived longer than males. Flies that partook of a partial blood meal were more viable than those that partook of a full meal. Flies that fed on ducks highly infected with the blood parasite, *Leucocytozoon*, were less viable than those fed on uninfected ducks, but in most cases flies, collected in the woods, that were not made to feed on ducks outlived those that fed on ducks. On the average, mortality increased during warm weather and decreased during cooler weather.

Introduction

The maintenance of adult female black flies in captivity is important in the study of the avian blood parasite, *Leucocytozoon*, which is known to be transmitted by a few species of black flies. Some black flies, such as *Simulium venustum* Say, apparently live two to three weeks in nature (5) although probably over 75% die sooner. The methods and results of keeping these insects alive in captivity during the summers of 1948–1950 in Algonquin Park, Ont., are given below.

Methods

Female black flies, 95% of which were *S. venustum*, were used in most experiments. In one experiment males were compared with females, and in another, females of *Simulium decorum* Walker predominated. Flies were collected by three methods. Those that had just emerged ('cage' flies) were collected from a screen cage placed over a neighboring stream. Those away from the stream ('wild' flies), were collected either by hand-netting or in vials as they landed on the observer. None of the cage flies had a blood meal, whereas most wild flies had a full or partial blood meal before being placed under artificial conditions.

The method of inducing wild flies to feed on specific birds is outlined below. Flies that landed on the observer were collected in 5-dram shell vials, usually from 5 to 10 flies per vial. The vial of flies was inverted as soon as possible over the bird's head, neck, back, or side, a small portion of which had been plucked or shorn of feathers. An opaque sleeve was placed over the vial and within $\frac{1}{4}$ to $\frac{1}{2}$ in. of the bird's skin, a procedure which took advantage of the positive phototaxis of the flies and drove them down to the skin. In this way many more flies were induced to feed, and in a shorter time, than without the sleeve. The flies that did not feed in a few minutes were released by removing the vial momentarily. When the vial was replaced, the sleeve was removed;

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and when the flies finished feeding, they flew up to the bottom of the vial. The flies could be made to stop feeding, if a complete meal was not desired, by bunting them gently with the lip of the vial until they let go and flew up into the vial. The vials were plugged with cotton and stored in a cool, dark place until the flies were transferred to the longevity tubes.

The longevity tubes consisted of 8-in. lengths of glass tubing (3-cm. bore) with cotton plugs in both ends (Fig. 1). These tubes were placed in jars containing $\frac{1}{4}$ in. of water or aqueous solution, the cotton plug being out of contact with the liquid. A capillary tube (3–4-mm. bore) with a cotton wick passed through the bottom plug, and dipped into the fluid so that flies were able to obtain moisture from the wick. Nutrient was added either in a small cheesecloth bag suspended from the top plug, in a receptacle pinned to the bottom plug, scattered on the bottom plug, or as a solution in the jar.

Results

Experiments were conducted to determine whether the longevity of the flies changed when the number per tube varied from 1 to 35 flies. A variation in the number per tube within this range was accompanied by no consistent increase in the rate of mortality (Table I). Therefore the number of flies per tube was allowed to vary from 1 to 35 during the experiments.

TABLE I

COMPARISON OF LONGEVITY IN TUBES CONTAINING DIFFERENT NUMBERS OF WILD FLIES, SURVIVAL BEING EXPRESSED AS A PERCENTAGE OF THE TOTAL ALIVE AFTER A GIVEN TIME

Number per tube	Total number of flies	Time interval in days							
		0	2	5	10	15	20	25	30
Unfed flies (with 10% sucrose solution)									
0– 9	11 (3)*	100	82	27	0				
10–19	109 (10)	100	69	20	4	1	0		
20–29	74 (3)	100	81	35	0				
Total, 0–29	194 (16)	100	74	26	2	0.5	0		
Unfed flies (with dry sucrose and water)									
0– 9	7 (1)*	100	86	43	14	0			
10–19	132 (8)	100	94	74	39	11	3	1	1
20–29	157 (6)	100	96	76	36	15	8	1	1
30–39	98 (3)	100	100	70	56	20	0		
Total, 0–39	394 (18)	100	96	74	42	15	4	1	1
Fed flies (with 10% sucrose solution)									
0– 9	147 (27)*	100	64	24	4				
10–19	645 (57)	100	66	24	0				
Total, 0–19	792 (84)	100	66	24	1.7				

* *Number of tubes in brackets.*

Experiments were conducted to discover the type of food that would give the greatest survival. In some tubes no food or water was added. In others, food in the form of sucrose crystals or cut raisins was provided, but no water. With water provided by the wick, the following nutrients were used: sucrose crystals, cut raisins, albumin, and sucrose crystals with albumin (10 : 1). Finally, different liquid media were used: water, solutions of 10%, 50%, and 100% saturation of C.P. dextrose and 'table' sucrose, 20% sucrose solution, 30% honey solution, 10% sucrose solution with 1% peptone, and dried albumin in the tube with 20% sucrose solution in the jar.

If no food or water was supplied, all wild flies died within two days; and similarily, if sucrose crystals or cut raisins were supplied without water, the flies died in two or three days. Wild flies, whether given an experimental blood meal or not, had a higher survival for two or three days with water alone than with any other medium, but thereafter there was a rapid die-off (Fig. 3). This indicates that black flies of this species must drink water to live, but that nutrient is needed for survival beyond three days. Dry albumin on the bottom plug with water in the jar gave a similar survival to that with water alone, but cut raisins and water separately gave a lesser survival throughout. Experiments with nutrient in solution indicated that sugar solutions of 10% saturation (at 70° F.) gave better survival of cage flies than 0%, 50%, or 100% (Fig. 2). Solutions of table sucrose gave a better survival than those of C.P. dextrose. For example, the percentage survival at five days using a 10% solution was 30% with sucrose and 11% with dextrose, and using a 50% solution, was 20% and 10% respectively. Thus sucrose was used in most of the experiments. More flies became bloated with solutions of 10% than with those of higher concentrations and the bloated flies died sooner than less swollen flies in the same tubes. Therefore, a 20% sucrose solution was tried and it gave a better survival of cage flies than the 10% solution after 15 days, but about the same as the 50% solution up to this time. The addition of 1% peptone to the sucrose solution did not increase longevity. A solution of 30% honey gave a slightly better survival of wild unfed flies than 20% sucrose solution, and both these gave a higher survival, except in the first two or three days, than water alone.

It was found that an improved longevity resulted when water only was placed in the jar and the sucrose was presented as dry crystals rather than in solution (Fig. 3). This was true whether or not the flies were fed previously on the blood of ducks. There was less tendency for the flies to become bloated when dry sugar rather than a sugar solution was used. The sugar was easiest to handle suspended in a bag from the top plug (Fig. 1). The sugar on the bottom plug or in the receptacle deliquesced and often flies became sticky; and in order to save them, they had to be freed from surfaces to which they adhered and sometimes washed in water. The longevity, especially of flies previously fed on ducks, was rather similar with these three methods of supplying dry sucrose. The addition of powdered albumin to sugar in the bag (1 : 10) slightly improved longevity over that with just sugar in the bag.

In most cases, however, the sugar scattered on the plug gave the highest survival of flies fed and not fed on blood, especially if there was not too much sugar at any one time.

The temperature during these experiments varied from 40–90° F. and averaged about 65° F. Comparison of longevity was usually made between tubes of flies maintained over the same period of time, however, and therefore under the same temperature conditions. In the course of the experiments it

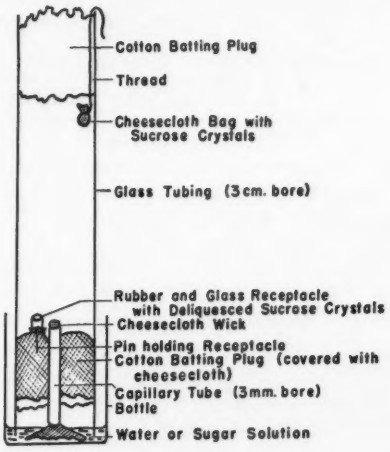

FIG. 1. Longevity tube for maintaining black flies in captivity. Note: The methods of presenting the food, namely, in the cheesecloth bag, in the receptacle, scattered on the bottom plug, and in solution, were each used in different experiments and are placed together on the diagram only for convenience.

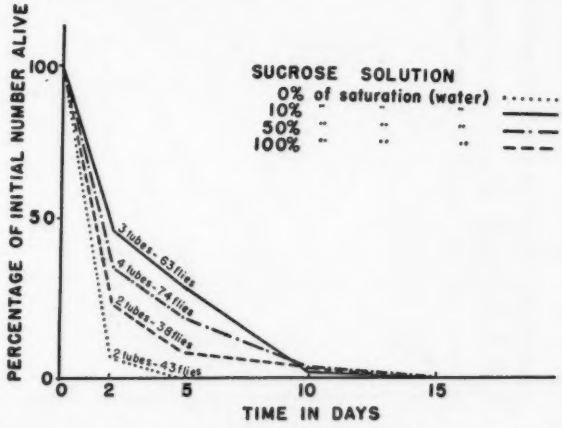

FIG. 2. A comparison of the longevity of female black flies that 24 hr. previously had emerged into the cage and were maintained on different concentrations of sucrose solution. These flies had no previous blood meal.

was observed that fewer flies died in the tubes during times of cool temperature. By comparing the percentage of each day's flies that died during the following 24 hr. with the mean temperature during that period (an average of the maximum and minimum temperatures), it was found that the mortality increased at high temperatures and decreased at low temperatures (Fig. 4).

The longest survival for any unfed *S. venustum* kept at outside temperatures during June and July was 37 days, and 18 (4%) lived for at least 20 days on

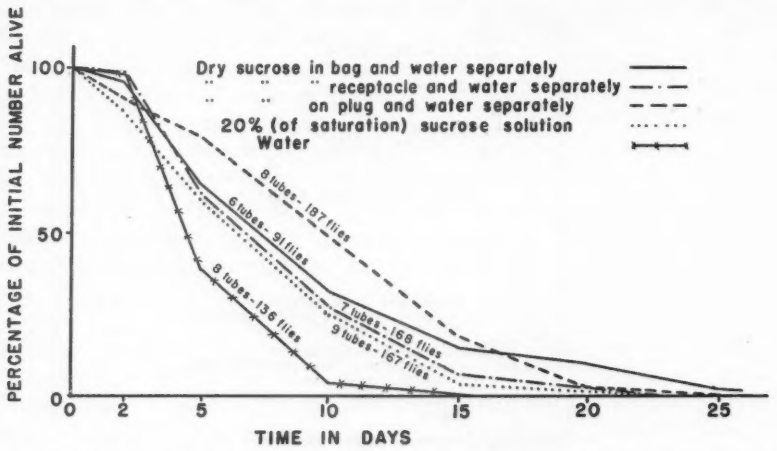

FIG. 3. A comparison of the longevity of female black flies collected at some distance from the stream and maintained on different media. These flies had no previous experimental blood meal.

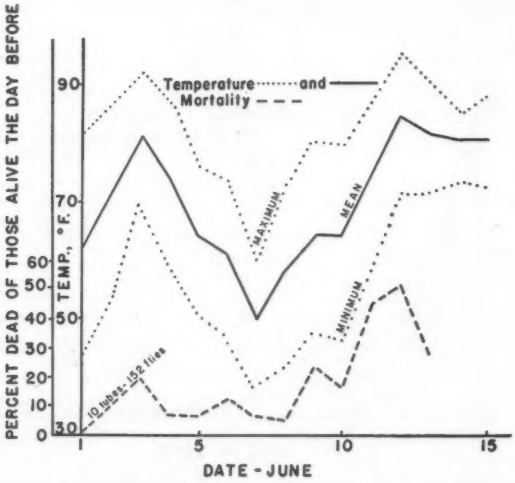

FIG. 4. The variation of the daily mortality of black flies in longevity tubes with air temperature near the tubes.

TABLE II

THE SURVIVAL OF BLACK FLIES MAINTAINED ON HONEY OR SUGAR SOLUTIONS OR ON SUCROSE CRYSTALS AND WATER SEPARATELY

	Time in days									
	0	2	5	10	15	20	25	30	35	40
Cage flies unfed, 20 tubes, 441 flies	100	37	17	4	0.5	0.2	0			
Wild flies unfed, 55 tubes, 983 flies	100	85	58	25	8	2	0.3	0.2	0.1	0
(21 tubes, 446 flies)*	(100)*	(96)	(70)	(38)	(13)	(4)	(0.5)	(0.5)	(0.2)	(0)
Wild flies fed, 49 tubes, 637 flies	100	73	35	15	4	1	0.4	0		
Total, 124 tubes, 2061 flies	100	71	42	18	5	1	0.3	0.1	0.1	0

* *A selection of only those tubes containing sucrose crystals and water.*

NOTE: *Unfed means no experimental blood meal, and fed means a partial blood meal from ducks infected with Leucocytozoon.*

dry sucrose and water (Table II). The results in the preceding paragraph suggested that keeping flies at a lower temperature would increase longevity. Several tubes of wild flies, with sucrose crystals in the suspended bag, that had been exposed to outside temperatures for eight days in mid-August, were placed in a refrigerator giving an average temperature of 52° F. In this way two female flies (*S. vittatum Zett.* and *S. decorum*) were maintained for 61 and 63 days, respectively, two females (*S. decorum*) for 35 days, and five for 33 days.

Most of these experiments were concerned only with females of *S. venustum*, but in one experiment the longevity of males of *S. venustum* was compared with that of females. Female flies were found to have a longer survival than male flies (Fig. 5).

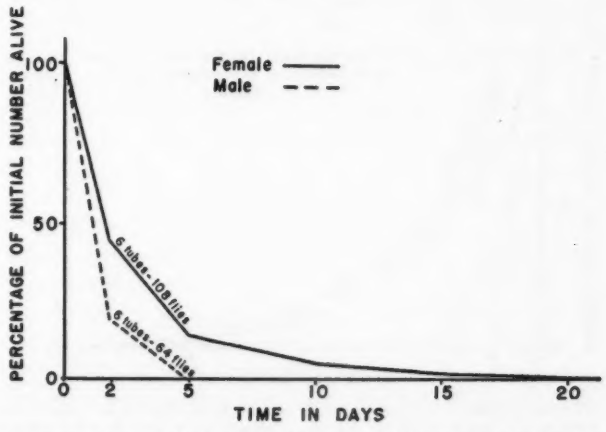

FIG. 5. A comparison of the longevity of male and female black flies that emerged into the cage 24 hr. previously. The flies had no blood meal, but both sexes were together in the same tubes, most of which contained dry sucrose and water.

Experiments were carried out to analyze the influence on longevity of the history of the flies prior to maintenance. A comparison between the longevity of cage flies that had emerged during the preceding 24 hr. and wild flies netted at points some distance from the stream indicated that the latter had a much higher survival (Fig. 6).

Some of the wild flies were induced to feed on domestic ducks. Experiments were conducted to determine the change in longevity with the size of a meal

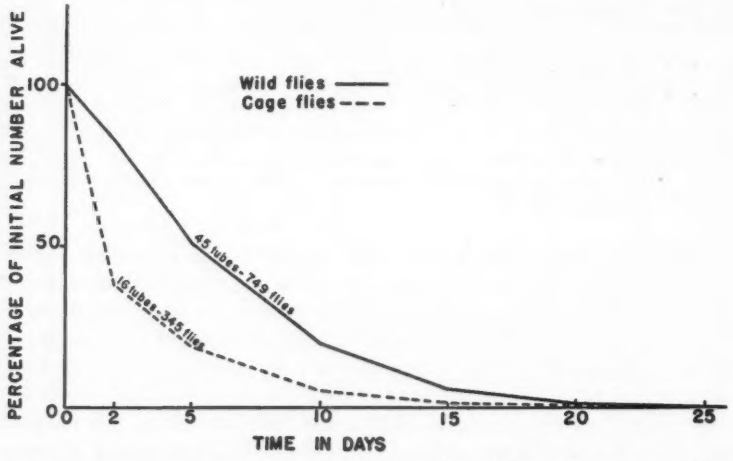

FIG. 6. A comparison of the survival of female black flies that 24 hr. previously emerged into the cage, and of those attracted to humans at some distance from the stream, including flies collected in 1948 and 1949. None of these flies were force-fed on ducks.

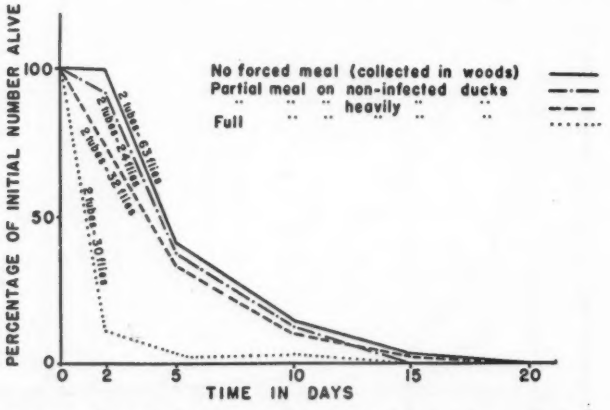

FIG. 7. A comparison of the longevity of female black flies that were collected away from the stream on June 11, 1949, some of which were fed on the blood of ducks infected with *Leucocytozoon* and others on the blood of noninfected ducks. These flies were maintained thereafter on sucrose crystals in a covered receptacle with water in the jar.

of blood infected with *Leucocytozoon*. Most black flies that partook of a full blood meal died within two days. Those that were given a partial blood meal lived longer but not as long as those that had no experimental blood meal (Fig. 7). Flies fed on uninfected blood of ducks lived longer than those fed on blood containing gametocytes of *Leucocytozoon* (Fig. 7).

Discussion

In nature some females of *S. venustum* live as long as three weeks from emergence to oviposition (5) and a few may live longer,* although many may die in much less time. Some that were captured at some distance from the stream had a few mature and many immature eggs in the ovary. These flies, which had presumably laid most of their mature eggs, were maintained in captivity for over a week after a preliminary meal on the blood of ducks, but the immature eggs in most cases, showed little development.

In captivity, females of *S. decorum* that were netted away from the stream and not given a preliminary blood meal were maintained for over 60 days on sucrose crystals and water. Wild females of *S. venustum* that were not given an experimental blood meal lived longer in a large number of tests than those that were (Table II), although in nature this species of black fly was observed feeding on the blood of mammals and birds. This difference in survival may be because of the increased handling received by the flies that are fed experimentally, as this difference was present whether the blood taken contained gametocytes of *Leucocytozoon* or not. However, longevity was reduced further when the blood was infected. That these parasites were developing in some, if not most, of these flies was demonstrated by the transmission of the infection to uninfected ducks by injecting into the ducks an emulsion of ground-up flies that had been fed on the same infected ducks and during the same period as the flies used in the longevity experiments (6). This reduction in longevity is similar to that found by Buxton (4) with *Culex fatigans* fed on the blood of canaries containing *Plasmodium* as compared to those fed on uninfected blood of canaries.

The fact that black flies that had emerged within the previous 24 hr. lived for a shorter time than those netted away from the stream (Table II) may have to do with selection, many of the newly emerged flies possibly being weak and predestined for an early death, whereas the wild flies are mainly a select group in which most of the weaklings have already died.

Other workers have had varying success in maintaining black flies in captivity. Jobbins-Pomeroy (9) kept a few reared specimens of *S. venustum* in bottles containing damp cloths, and they lived for five days without food. Females that had just finished ovipositing were maintained for three days. Friederichs (7) succeeded in keeping two females of *S. maculatum* Mg. alive on sugar solution for 23 days in a large cage; the majority died within 14 days.

** Dalmat in Guatemala (Ann. Entomol. Soc. Am. 45 : 33, 1952) collected, marked, and released 28,490 individuals of Simulium metallicum Bell. Recaptures of those released indicated that 35 lived for over 20 days, 8 for over 60 days, and 1 for at least 85 days.*

Blacklock (3) kept one out of 377 individuals of *S. damnosum* Theob. alive for as long as 19 days, two-thirds dying in about a week. Wu (11) experimented with specimens of *S. vittatum* and *S. jenningsi* Mall. and found the greatest longevity with a water diet was six days, most dying within three days. However, the period was greatly lengthened for both males and females by feeding them on sugar solution or soaked raisins, two males and three females of *S. vittatum* and two females of *S. jenningsi* living for 18 days, but the majority dying within eight days. In general, she found that the female outlived the male. Bequaert (2) was able to keep only a few specimens of *S. metallicum* Bell. alive for six days, the majority dying within three days. Baranov (1) reported a longevity in black flies of 20–30 days at a relative humidity of 90%. Using an apparatus designed by Gebert (8) and somewhat similar to the longevity tube of the present study, Wanson *et al.* (10) maintained females of *S. damnosum* for as long as 11 days. This shows a wide variation in the period of survival, part of which may be caused by method and part by the species of black fly.

Acknowledgments

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Contents

Page

The Desoxyribonucleic Acid Content of the Liver Nuclei of Immature Pullets Treated With Gonadal Hormones—*W. E. J. Phillips, W. A. Maw, and R. H. Common* - - - - - - - - - - - - - - - - 167

A Cytological Study of the Anterior Submaxillary Glands of the Fowl, *Gallus domesticus*—*D. J. McCallion and H. E. Aitken* - - - - - - - - 173

Postreduction of the *X*-chromosome and Complete Chiasma Interference in the Lampyridae (Coleoptera)—*Stanley G. Smith and Doreen E. Maxwell* - - 179

The Limnology of Amethyst Lake, a High Alpine Type Near Jasper, Alberta—*D. S. Rawson* - - - - - - - - - - - - - - - - 193

Variation in Anal Fin Ray Count of the Redside Shiner *Richardsonius balteatus* (Richardson)—*C. C. Lindsey* - - - - - - - - - - - - - - 211

The Salivary Gland Chromosomes of the Black Fly *Simulium vittatum* Zett.—*K. H. Rothfels and R. W. Dunbar* - - - - - - - - - - - - 226

The Nature of Cycles in Populations of Canadian Mammals—*L. Butler* - - 242

The Ptilinal Armature of Flies (Diptera, Schizophora)—*E. H. Strickland* - - 263

The Effect of Metamorphosis on the Multiplication of an Insect Virus—*F. T. Bird* - - - - - - - - - - - - - - - - 300

Longevity of Black Flies in Captivity—*Douglas M. Davies* - - - - - - 304